Non-invasive cardiac imaging and the risk of coronary atherosclerosis

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Non-invasive cardiac imaging and the risk of coronary atherosclerosis

Niet-invasieve cardiale beeldvorming en het risico op coronair lijden

(met een samenvatting in het Nederlands)

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To my parents and Marisevi

In memory of Guido Lankamp

27-8-1975 / 17-3-2002

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Chapter 1

General Introduction

General introduction

Coronary heart disease (CHD) is among the most common causes of death in our society and the incidence of acute myocardial infarction is very high[1;2]. Almost half of the patients with a first acute myocardial infarction die before reaching the hospital and therefore focus should be on prevention[1].

In this thesis, we focus on the application of non-invasive cardiac imaging modalities, such as multi-detector computed tomography (MDCT) in asymptomatic patients at high risk for cardiovascular events and its role in assessing epicardial adipose tissue, a potential risk factor for the development of coronary atherosclerosis.

Non-invasive cardiac imaging

In recent years MDCT has been evolving at a very high rate[3]. Temporal resolution has improved with the use of faster X-ray tube rotation. In addition, the use of thinner detectors has increased spatial resolution. Several studies show the high accuracy of MDCT to detect clinically important coronary stenoses[4-6]. Rupturing coronary plaques are often non-calcified plaques that cause a moderate stenosis of less than 50% and are not detectable with conventional angiography[7;8]. With MDCT a distinction can be made between non-calcified, mixed and calcified coronary plaques[9-12]. Screening for silent coronary atherosclerosis has become an option with recent non-invasive developments in MDCT and dobutamine stress magnetic resonance (DSMR) testing[13-17]. Coronary calcification is a sign of coronary atherosclerosis. Quantification of these calcium deposits inside the vessel wall with the use of MDCT (calcium scoring) plays an important role in cardiovascular risk assessment[18].

Screening in cardiac asymptomatic high risk patients

Peripheral arterial disease (PAD) is a prevalent disease in the western world[19], especially in the elderly. It is associated with a considerably increased risk of future cardiovascular events due to the presence of generalized atherosclerosis in these patients and most will die from CHD[20-23]. Risk factor management guidelines state that patients with PAD should receive optimal risk factor treatment, i.e. aspirin, a statin and antihypertensive medication, if indicated and counseling with regard to

physical exercise, healthy diet and cessation of smoking[24]. Screening asymptomatic patients for the presence of coronary atherosclerosis appears feasible[12;25]. Guidelines also state that a significant stenosis in the left main coronary artery is a class 1 indication for revascularization in cardiac asymptomatic patients [26].

In **chapter 2** we describe the rationale and design of the GROUND study, a multi-center randomized clinical trial, set up to evaluate the use of these modern non-invasive imaging modalities in a screening algorithm to detect severe coronary atherosclerosis in patients with PAD, who do not have symptomatic cardiac disease, in reducing vascular morbidity and mortality. In **chapter 3** we present baseline results from the GROUND study. In this chapter we focus on the occurrence of treatable coronary artery disease; significant stenoses in the left main coronary artery or its equivalent. Next, in **chapter 4** we describe the burden of coronary artery plaque in the GROUND study. It is known that coronary atherosclerosis is prevalent in this population of cardiac asymptomatic patients with PAD [27]. However, the extent of coronary atherosclerosis and the distribution of different types of coronary plaques in this patient group has not been described before.

PAD patients are expected to be in the highest calcium scoring risk group due to the presence of atherosclerosis in their peripheral arteries. It is unknown if calcium scoring can be of use in high-risk cardiac asymptomatic PAD patients to further differentiate risk. In **chapter 5** we study coronary calcification in this patient group in relation to other cardiovascular risk factors and we determine whether the amount of coronary calcification on top of risk factors may be of help in predicting the occurrence of a significant left main (or equivalent) coronary artery stenosis, eligible for bypass surgery[26].

Epicardial adipose tissue and coronary artery disease

Studies show that visceral adipose tissue is an important indicator of cardiovascular risk [28-31]. Epicardial adipose tissue (EAT) is a layer of visceral fat between the myocardium and the pericardium [32]. EAT, as well as intra-abdominal fat, appears to originate from the same brown adipose tissue of infancy and is a rich source of bioactive molecules in the direct surrounding of the coronary arteries[33]. In vivo studies show that chronic adventitial treatment with bioactive molecules induces coronary intima lesions [34-36]. It is conceivable that EAT contributes to the local

development of atherosclerosis and the occurrence of cardiovascular events. The few studies to date that assessed EAT predominantly used echocardiography, demonstrating that right ventricular EAT is related to several known cardiovascular risk factors, including waist circumference, blood pressure, left ventricular mass and high levels of insulin[28;37;38]. In addition, the amount of all the adipose tissue (peri- and epicardial) surrounding the heart has been related to the severity of coronary atherosclerosis, assessed by coronary angiography[39]. Because the pericardium can be presumed to hamper the free diffusion of bioactive molecules, the EAT and, in particular, the adipose tissue directly surrounding the coronary arteries rather than all the adipose tissue surrounding the heart should affect the development of coronary atherosclerosis. However, the relation between only the EAT that directly surrounds the coronary arteries and coronary atherosclerosis has not yet been addressed.

We developed a method for measuring this peri-coronary EAT with cardiac MDCT and in **chapter 6** we describe and compare various methods for quantifying EAT and peri-coronary EAT. On calcium scoring MDCT scans of the heart, peri-coronary EAT is easily detected. In **chapter 7** we set out to study whether peri-coronary EAT is associated with cardiovascular risk factors and coronary artery calcification. The metabolic syndrome is a combination of risk factors, consisting of hypertension, high fasting plasma glucose, low HDL cholesterol and increased waist circumference [40]. Individuals with metabolic syndrome are at substantially increased risk of developing CHD[41-44]. Many common cardiovascular risk factors are associated with EAT [28;37;38]. However, it is unclear whether the metabolic syndrome is associated with an increased prevalence of peri-coronary EAT. In **chapter 8** we explore the relation between peri-coronary EAT and the metabolic syndrome. In **chapter 9** we explore the relation between volume and thickness of EAT, as measured with MDCT, with the extent of coronary atherosclerosis in patients referred for coronary angiography. The results of the studies presented in this thesis are summarized and discussed in **chapter 10**. The results are looked at in a larger perspective and ideas are given for future direction of studies in this field.

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Chapter 2

Rationale and Design of the GROUND study: Non-invasive Cardiac Assessment in High Risk Patients

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Trials. 2008;9:49.

Abstract

Background: Peripheral arterial disease (PAD) is a common disease associated with a considerably increased risk of future cardiovascular events and most of these patients will die from coronary artery disease (CAD). Screening for silent CAD has become an option with recent non-invasive developments in CT (computed tomography)-angiography and MR (magnetic resonance) stress testing. Screening in combination with more aggressive treatment may improve prognosis. Therefore we propose to study whether a cardiac imaging algorithm, using non-invasive imaging techniques followed by treatment will reduce the risk of cardiovascular disease in PAD patients free from cardiac symptoms.

Design: The GROUND study is designed as a prospective, multi-center, randomized clinical trial. Patients with peripheral arterial disease, but without symptomatic cardiac disease will be asked to participate. All patients receive a proper risk factor management before randomization. Half of the recruited patients will enter the 'control group' and only undergo CT calcium scoring. The other half of the recruited patients (index group) will undergo the non invasive cardiac imaging algorithm followed by evidence-based treatment. First, patients are submitted to CT calcium scoring and CT angiography. Patients with a left main (or equivalent) coronary artery stenosis of > 50% on CT will be referred to a cardiologist without further imaging. All other patients in this group will undergo dobutamine stress magnetic resonance (DSMR) testing. Patients with a DSMR positive for ischemia will also be referred to a cardiologist. These patients are candidates for conventional coronary angiography and cardiac interventions (coronary artery bypass grafting (CABG) or percutaneous cardiac interventions (PCI)), if indicated. All participants of the trial will enter a 5 year follow up period for the occurrence of cardiovascular events. Sequential interim analysis will take place. Based on sample size calculations about 1200 patients are needed to detect a 24% reduction in primary outcome.

Implications: The GROUND study will provide insight into the question whether non-invasive cardiac imaging reduces the risk of cardiovascular events in patients with peripheral arterial disease, but without symptoms of coronary artery disease.

Background

Peripheral arterial disease and coronary artery disease

Peripheral arterial disease (PAD) is the term used to refer to lower-extremity arterial disease. It is a sign of systemic atherosclerosis affecting millions of people, in particular the elderly. Reports from the Framingham Heart Study suggest that the prevalence of PAD has increased between 1970 and 2003 [1,2]. Estimates are that approximately 10% of individuals > 55 years have asymptomatic PAD (defined as an ankle-brachial index (ABI) < 0.90) [3]. The prevalence of so called intermittent claudication (IC) in patients aged 55 to 74 years is approximately 4.6% [3] and the prevalence of pain at rest and necrotic lesions (Fontaine stage IV) is approximately 1% [4]. Despite the relatively benign prognosis for the affected limb, symptoms of IC should be regarded as a sign of systemic atherosclerosis and a high risk of cardiovascular events. In a review on IC, Coffman et al. [5] described survival rates among IC patients of approximately 70% to 80% after 5 years, 40% after 10 years, and 26% after 15 years. More recent studies showed an overall 5-year-mortality rate of 19.2% vs. 10% in controls [6] and 10-year-mortality rates of 61.7% among male and 33.3% among female patients with IC, compared to 16.9% of men and 11.6% of women without evidence of PAD [7]. The mean age of participants in these studies was 67 and 66 years, respectively. Mortality due to coronary artery disease (CAD) after 5 years in a study by Leng et al. was 5.5% vs. 2.6% in controls [6] and after 10 years cardiac death occurred in 35.3% of men and 9.1% of women with IC, compared to 5.5% of men and 2.2 % of women without IC [7]. So not only do PAD patients have two or three times the overall mortality, the risk of cardiac death is even 4-6 times higher [7]. This increase in cardiovascular mortality is not surprising since several studies showed a two or three times increase in cardiovascular morbidity in PAD patients [6;8;9]. In 2003 Sonecha et al. published a study in which they found CAD in 46% of IC patients, compared to 6% in controls; 31% of claudicants even had 2-/3-vessel disease [10]. Aronow et al. found a prevalence of CAD in PAD patients of 58% [11]. Hertzer et al. described the results of coronary angiography (CAG) in 1000 patients scheduled for vascular surgery. In this group, 381 had complaints of lower extremity ischemia, of whom 166 (44%) had no cardiac complaints. CAG revealed the presence of CAD in 86% of these cardiac asymptomatic PAD patients [12]. Therefore, assessment of cardiac atherosclerotic abnormalities using non-invasive techniques followed by appropriate

treatment may help to improve survival in patients with PAD but yet without cardiac symptoms.

Cardiac imaging with multi-detector CT and MRI

Since the discovery of selective coronary angiography (CAG) by Sones in 1958, it has been the method of choice for detection and follow-up of CAD. Several studies have shown that diagnostic CAG has a morbidity of 2% and a mortality of approximately 0.1% [13-15]. For screening purposes non-invasive imaging would be much more suitable. The rapid development of multi-detector computed tomography (MDCT) has made it possible to image the heart and its coronary arteries in a non-invasive way. It is much faster than older scanners and images are obtained with sub millimeter spatial resolution and high temporal resolution. As a result of simultaneous recording of an electrocardiogram (ECG) signal, several image reconstructions are possible in different phases of the heart cycle. [16]

Several studies showed the high accuracy of MDCT to detect clinically important coronary stenoses. [17-19] Not only the costs and risk of complications are lower with MDCT than with CAG [13-15], this technique also has the advantage of vessel wall visualization. Both the composition of the plaque and its impact on the vessel lumen can be detected. A distinction can be made between lipid, fibrous and calcified coronary plaques [20]. In recent years it has become clear that plaque composition may be a better risk-predictor for acute coronary events than stenosis grade. Rupture of so called vulnerable plaques accounts for approximately 70% of sudden coronary deaths.[21] Although the absolute risk of severely stenotic plaques may be higher than the absolute risk of mildly stenotic plaques, the number of plaques with mild stenoses overwhelmingly exceed the number of plaques with severe stenoses [21]. Dobutamine stress cardiovascular magnetic resonance imaging (DSMR) is used to identify wall motion abnormalities of the left ventricle indicative of myocardial ischemia [22-26]. It has been shown to be an accurate and safe diagnostic modality to assess myocardial ischemia and viability in patients with proven or suspected CAD [23-28]. A study by Nagel et al. showed that the presence of myocardial ischemia can be detected more accurately with DSMR than with dobutamine stress echocardiography (DSE). Image quality of DSMR is higher and with MRI sensitivity increased from 74.3% to 86.2% ($P<0.05$) and specificity increased from 69.8% to 85.7% ($P<0.05$)

compared to echocardiography [23]. With the use of myocardial tagging sensitivity can be increased up to 96% [26]. In this study by Kuijpers et al. the cardiovascular occurrence-free survival rate was 98.2% after a negative DSMR during a mean follow-up of 17.3 months. Furthermore, MRI allows optimal detection of dysfunctional, but viable myocardium. This is of clinical importance since revascularization of dysfunctional, but viable myocardium may improve left ventricular function and thus prognosis [29].

In patients with non-specific symptoms of coronary artery disease DSMR can be used to assess risk levels for coronary events with high accuracy. In a group of 100 patients suspected of coronary ischemia Van Dijkman et al. found a positive predictive value (PPV) of 98% and also a negative predictive value (NPV) of 98% for ischemia with DSMR. In this study the prevalence of ischemia was 43% [30]. In another study by Hundley et al. a 97% cardiac event free survival rate in 103 patients suspected of ischemia with a negative DSMR was observed [25]. Patients with a negative DSMR without rest wall motion abnormalities (RWMA) and without a history of CAD have an excellent cardiac prognosis and can be excluded from further clinical follow-up [31].

Compared to other non-invasive techniques, DSMR may be a valuable adjunct for the assessment of patients with (suspected) ischemic heart disease [32].

Treatment of silent coronary artery disease

According to the guidelines CABG or PCI may be considered as first line therapy in case of severe abnormalities in the coronary artery tree, even in asymptomatic patients [33]. Absence of cardiac symptoms should not be regarded as a sign of a more benign process.[34] In addition, silent myocardial ischemia has been shown to increase coronary artery disease risk and evidence indicates that in certain groups of these patients CABG or PCI treatment may reduce the risk. The results of the Asymptomatic Cardiac Ischemia Pilot (ACIP) study indicate that higher-risk patients with asymptomatic ischemia and clinically important coronary artery abnormalities, who undergo revascularization with CABG or PCI may have a better outcome as compared to those only receiving medical therapy [35]. Studies on the treatment of silent ischemia are all conducted in small groups of patients with coronary abnormalities [36]. In patients with left main disease, the survival benefit of CABG

compared to medical therapy is 19.3 months at 10-year follow-up. Therefore, the benefit of surgery over medical treatment for patients with left main stenosis (> 50%) is little argued [37] .

Study objective

This prospective, randomized, controlled, multicenter trial is designed to evaluate whether a cardiac imaging algorithm using non-invasive imaging techniques followed by evidence based treatment will reduce the risk of cardiovascular disease in cardiac asymptomatic patients with peripheral arterial disease. This imaging algorithm consists of coronary calcium scoring, MDCT angiography, and dobutamine stress MRI. Participants will be followed up for a period of five years (figure 1). As a secondary objective we will explore the role of coronary calcification in this particular patient group.

Methods

Study group

The study group will consist of approximately 1200 patients with peripheral arterial disease without a history of symptomatic cardiac disease. Patients are recruited from the vascular surgery departments of the participating centers (appendix 1). The study is in compliance with the Helsinki Declaration and local ethics committees gave their approval. Patients willing to participate will be asked to sign the informed consent form. Patients are eligible if they are 50 years or older and have peripheral arterial disease, at least stage Fontaine II, as diagnosed by the vascular surgeon. Patients will be considered not eligible for the study if they meet one of the following exclusion criteria: physician diagnosed history of symptomatic cardiac disease; cardiac rhythm other than sinus; unable to sustain a breath-hold for 25 seconds; asthma; contra-indications to MRI examination, such as vessel clips in the brain, metal splinters in the eye, insulin pump or other electronical devices that cannot be removed easily, metal implants, port-a-cath catheter or claustrophobia; contra-indications to iodinated contrast agent; severe arterial hypertension (> 220/120 mmHg); clinically important aortic stenosis; unable to remain in supine position for at least 60 minutes; extreme obesity (BMI > 40 kg/m^2); renal insufficiency (serum creatinine level

exceeding 140 μmol/l); severe physical deterioration due to concomitant disease; language barrier; and contra-indications to dobutamine.

Baseline risk factors

At baseline, eligible patients complete a questionnaire on current medication use, risk factors and quality of life. Height, weight, blood pressure and ankle pressure for calculation of the ankle-brachial index will be measured in the outpatient clinic. Total cholesterol, high density lipoproteins (HDL), triglycerides, creatinine, homocysteine, glucose and high sensitivity c-reactive protein will be measured at the local laboratory. These measurements were not standardized across the four different laboratories.

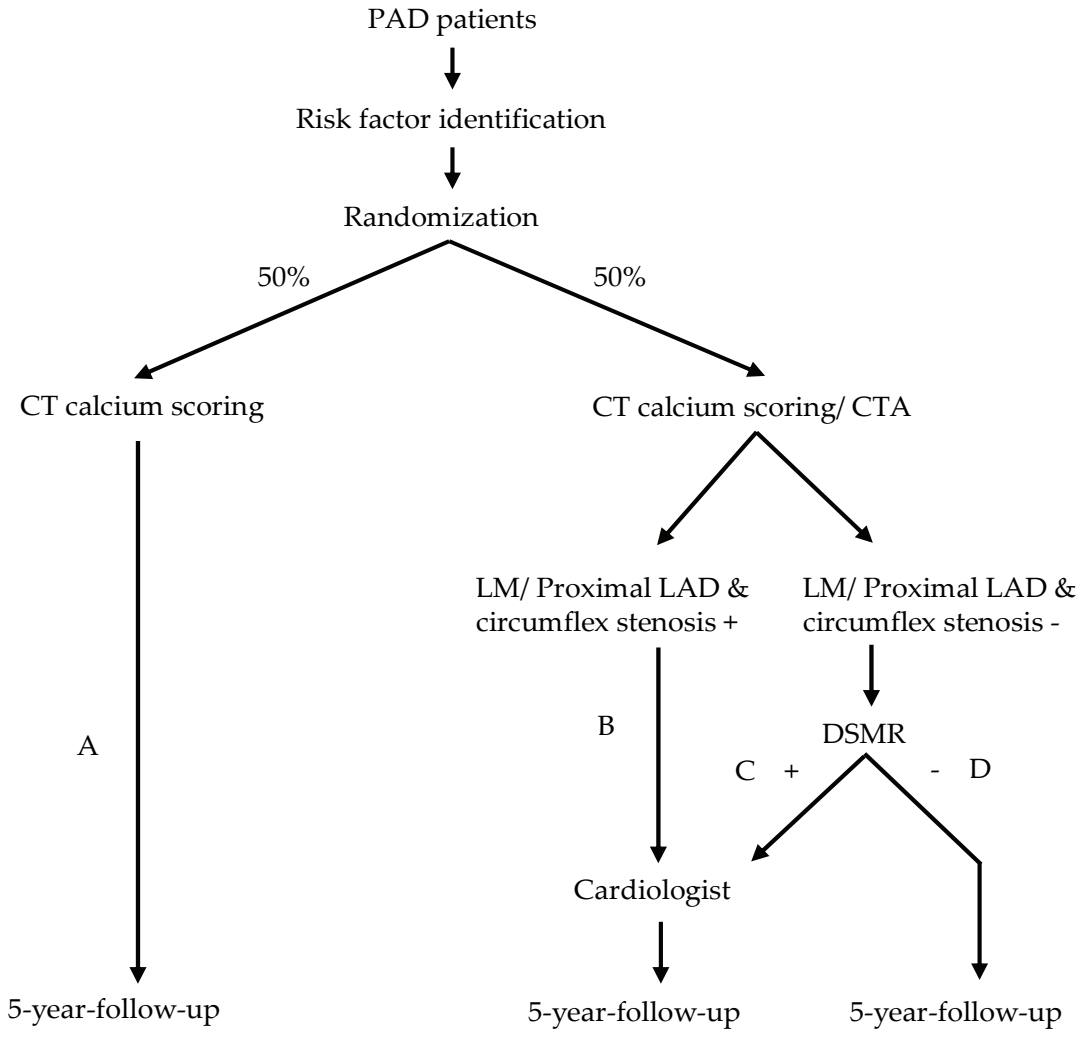

Figure 1. Flow Diagram of the GROUND Study.

To prevent further progression of their present cardiovascular disease all patients will be treated according to the Dutch guidelines for treatment of atherosclerotic peripheral arterial disease [38]. These guidelines state that these patients should receive aspirin, a statin and antihypertensive medication, if indicated. Patients also receive proper advice regarding exercise, healthy diet and cessation of smoking.

MDCT imaging

All centers participating in the GROUND study are experienced in making cardiac MDCT angiography scans and use at least a 16-slice CT scanner. The patient preparation will start with explanation of the procedure. The ECG monitor is connected and sinus rhythm is monitored for 1 minute. Then the patient practices a 25 second breath-hold. The MDCT calcium scoring examination will follow a scout view. It will be done either prospectively ECG-triggered or retrospectively gated according to the local hospital protocol. For the patients randomized to the imaging arm of the study, calcium scoring will be followed by the contrast enhanced retrospectively ECG-gated CT angiography. An 18 G intravenous line is started. Patients who have a heart rate over 60 bpm will be administered i.v. beta-blockers (a minimum of 5 mg metoprolol). Patients will continue to receive beta-blockers until their heart rates are below 60 bpm, or 20 mg has been administered. Blood pressure will be monitored. The contrast volume and infusion rate will be calculated individually depending on patient weight and scan duration and contrast concentration. Ten axial image datasets will be reconstructed every 10% of the RR interval on the electrocardiogram.

Total examination time for CT calcium scoring will be approximately 10 minutes and the total examination time for CT calcium scoring and CT angiography, including preparation of the patient, will be approximately 30 minutes. Total estimated radiation dose for the patient is recorded.

MDCT Analysis

The cardiac MDCT data will be analyzed by the site investigator at the site. The reader will use the appropriate workstation for all data analysis. The site investigator is responsible for incidental abnormal findings in the dataset.

The calcium scoring study will be evaluated using an established analysis program (Heartbeat-CS, EBW, Philips Medical Systems). Agatston, Mass and Volume scores will be determined and recorded on case report forms. For the MDCT angiography the site investigator will identify the phase with the least amount of cardiac motion. This phase is then loaded into the appropriate application. Depending on the coronary morphology and quality of the scan several post processing techniques will be applied to assess the coronary arteries. The dataset will be evaluated in terms of contrast opacification, assessability, stenoses and plaques. The proximal, medial and distal coronary arteries and cranial, medial and caudal right ventricle are evaluated for contrast opacification and image noise using a 5-point scale (1=non-diagnostic, 2=limited diagnostic, 3=acceptable, 4=good, 5=excellent).

The 15-segment tree from the American Heart Association will be used for segment definition. Each segment will be evaluated for assessability using a 5-point scale (1=non-diagnostic, 2=limited diagnostic, 3=acceptable, 4=good, 5=excellent). For the segments that are not assessable (score 1 or 2) the reader will indicate why the segment cannot be evaluated according to the following choices: (0) anatomical reason, (1) respiratory motion, (2) cardiac motion, (3) arrhythmia, (4) calcium, (5) vessel size (small caliber), (6) poor opacification, (7) streak artifacts, (8) scan range, (9) noise, (10) technical failure. Any luminal narrowing greater than 30% will be visualized from the curved MPR and will be quantified according to a 4-point scale: (1) 30-50%, (2) 50-70%, (3) 70-99% and (4) 100%. The type of visualized plaque will be indicated as soft, calcified or mixed. All scan data will be transferred to the core laboratory for a second reading of the data. Inter- and intra-observer variability will be determined.

DSMR imaging

Patients randomized to the imaging group will under go dobutamine stress MRI within three weeks of the MDCT angiography. All beta-blocking medication will be stopped 4 days prior to the examination[26]. After instructions by the technician, the patient is positioned on the scanning table and an intravenous access will be established through an antecubital vein. ECG leads, a phased-array surface coil covering the heart, and a brachial blood pressure cuff are applied. During the procedure, a single-lead ECG will be continuously monitored. Systolic blood

pressure, diastolic blood pressure and heart rate will be recorded at baseline and every three minutes throughout the procedure.
Baseline imaging will consist of acquiring three short-axis cine images (basal (1.5 cm below mitral valves), mid-ventricular and apical) and one vertical long-axis cine image. Cine tagged images are made in the three short axis planes.

During the DSMR dobutamine will be infused intravenously using a digital pump injector situated outside the scanner room. The dose will be increased to 10, 20, 30 and 40 μg/kg/min with a six minutes time interval. In case of rest wall motion abnormalities (RWMA), infusion will be started at 5 μg/kg/min. Image acquisition will start three minutes after each dose increment. Imaging will consist of acquiring three short-axis cine images (basal, mid-ventricular and apical) and one vertical long axis with and without myocardial tagging. Criteria for ending the examination are (1) development of new or worsening wall motion abnormalities (NWMA) in more than 1 myocardial segment, (2) fall of systolic blood-pressure of > 40 mmHg, (3) marked hypertension > 240/120 mmHg, (4) severe chest pain, (5) complex cardiac arrhythmia's and (6) intolerable side effects of dobutamine.

Both a radiologist (or a trained radiology resident) and a cardiologist (or a trained cardiology resident) will be present in the MR suite to monitor the condition of the patient and to directly evaluate the images. The target heart rate rule is not applied. Studies have shown that this is a safe and effective method [26;28;31] .

All participating centers have experience in dobutamine stress testing. Although side effects are rare, a protocol to remove the patient from the scanner room in case of an emergency is practiced regularly. Total examination time for a DSMR study, including preparation of the patient, will be approximately 50 minutes.

DSMR Analysis

The DSMR data will be analyzed by the site investigator at the site. The reader will use the appropriate workstation for all data analysis. For image interpretation multiple cine loop display will be used displaying at least three different stress levels for each slice simultaneously. Per segment wall motion will be graded using a 4-point scale according to the guidelines of the American Society of Echocardiography (1= normal or hyperkinesia, 2= hypokinesia, 3= akinesia and 4= dyskinesia). The sum of points is divided by the number of analyzed segments and yields the wall motion

score. [39] Normal contraction results in a wall motion score of 1, a higher score is indicative of wall motion abnormalities. During dobutamine stress with increasing doses, a lack of increase in either wall motion or systolic wall thickening, a reduction of both or significant changes in the rotational pattern of left ventricular myocardium ('tethering') are indicative of pathological findings. Myocardial ischemia will be defined as an induced WMA in at least two segments at consecutive planes of the left ventricle. RWMA will be defined as WMA in one or more segments at baseline. If RWMA's are present, which improve during low-dose dobutamine stress, but worsen during peak-stress, this will be considered diagnostic of inducible myocardial ischemia. If RWMA's are present, which do not improve with low-dose dobutamine, this will not be considered diagnostic of inducible ischemia. Also ejection fraction, end-diastolic volume and end-systolic volume will be documented. All scan data will be transferred to the core laboratory for a second reading of the data. Inter- and intra-observer variability will be determined.

Randomization

Randomization will be performed per hospital to ensure an equal distribution of groups of patients within hospitals. Directly after the patient gives informed consent, he or she is randomized with the use of the randomization module at the GROUND website. Only the data management center is aware of block size. This way half of the patients will be randomized for the imaging-with-treatment algorithm (groups B, C and D, figure 1), the other half of the patients (group A) undergoes only CT calcium scoring and enters follow-up. Patients randomized for the treatment groups (groups B, C and D, figure 1) will be scheduled for MDCT angiography. If a stenosis of the left main coronary artery (LM) (or equivalent) of more than 50% is observed on the CTA of a patient in the imaging-with-treatment group, he/she will be referred to a cardiologist for further diagnosis and treatment (group B, figure 1). A stenosis in the proximal left anterior descending coronary artery (LAD) in combination with a stenosis in the proximal circumflex coronary artery (LCX) is considered equivalent to a LM stenosis. All other patients in this group will undergo DSMR testing (groups C and D). Patients with a DSMR positive for ischemia are referred to a cardiologist for further diagnosis and treatment (group C). Further diagnostics and treatment will be left up to the cardiologist.

Data collection

Study data, including detailed data on diagnostic and therapeutic measures taken by the cardiologist, will be collected on case report forms (CRF's) and submitted on line to the data management center, located at the Julius Center for Health Sciences and Primary Care (www.juliuscenter.nl), where all forms are reviewed for completeness. CRF's are available on the GROUND website. Data will end up in a dedicated database.

End points and follow-up

All patients will be asked to fill out a short follow up form every half year for a total period of five years. The occurrences of events are recorded. The quality of life assessment is based on the SF36 questionnaire and repeated after 12, 30, 48, and 60 months. This questionnaire has been validated in the Dutch population [40] . Endpoints of the GROUND study are in concordance with the SMART[41] study endpoints.
The term 'end points' is used to describe death, cardiovascular complications, and interventions. Apart from death the occurrence of an endpoint does not imply that the follow-up will be ended. Endpoints in the GROUND study are summarized as MACE: Major Adverse Clinical Events. Primary outcome is a composite endpoint comprising fatal and non-fatal myocardial infarction and stroke, and vascular death (death due to vascular disease). Secondary end points are: fatal and non-fatal myocardial infarction; fatal and non-fatal stroke; vascular interventions; amputation; aortic rupture; end stage renal failure extra cranial hemorrhage; complications of CABG or PCI and all cause mortality.

Reported endpoints are classified by the Endpoint Committee, which is unaware of the randomization allocation. Clinical information (letters of discharge) is obtained from the treating specialist or general practitioner. All reported endpoints enter an endpoint verification procedure. Copies of discharge records are sent to the members of the Endpoint Committee. The members of the Endpoint Committee do not share the information between each other, but classify the events independently. Only if discharge records are inconclusive further medical information is obtained (results from laboratory findings, copy of the ECG, copies of imaging reports). The classifications are compared. If two members do not agree the endpoint will be

discussed with the blinded research physicians of the GROUND study group. They will decide or consult an extra physician, whose judgment is regarded as final.

Sample size considerations

The sample size is determined by the estimated risk in the group of patients randomized to usual care (control group, A, figure 1) and the risk observed in the groups that undergo cardiac imaging followed by subsequent treatment by a cardiologist as outlined in the protocol (groups B to D). Based on earlier studies in the Netherlands the 5-year-risk in the control group is assumed 24%[42]. The 5 year risk of cardiovascular morbidity and mortality among the IC patients that undergo cardiac imaging and subsequent treatment is based on the sum of **I)** the risk observed in those with stenosis in the main left coronary artery (group B, figure 1) plus **II)** the risk observed in those with limited vessel disease but no ischemia during the dobutamine stress test (group D) plus **III)** the risk observed in those with limited coronary vessel disease but with ischemia during the dobutamine stress test (group C).

The prevalence of these subgroups B to D in the arm of cardiac imaging and subsequent treatment is estimated to be 8% for B; 70% for D and 22% for C. Using the literature we have estimated the risk of cardiovascular morbidity and mortality for these groups of patients belonging to category B to D.

The 5-year risk of cardiovascular morbidity and mortality in these subgroups is 66% for those with stenosis of the left main coronary artery[41] (group B), 32.8% for those with cardiac ischemia during the stress test (group C) and 16.4% for those without cardiac ischemia (group D).

The estimates for patients in categories C and D are based on the assumption that those with cardiac ischemia have a doubling of risk compared with those without cardiac ischemia and that the risk of all groups combined should add up to 24%.

The effects of interventions performed by the cardiologist on the risk observed in PAD patients who undergo cardiac imaging are based on published international data from the ACC and AHA guidelines. These effects are for the two appropriate subgroups: 70% reduction in 5-year event rate using reperfusion therapy (PCI/CABG) for group B and 40% reduction in 5-year event rate using reperfusion

therapy (PCI/CABG) for group C. Patients without cardiac ischemia (group D) will receive no treatment.

Based on these estimates, the 5-year risk of cardiovascular morbidity and mortality will be 17.4 % in the intervention group (the combined risk for groups B and C; 70% event reduction in the group with a risk of 66% applying to 8% of the population; 40% event reduction in the group of patients with a risk of 32.8% applying to 22% of patients), reflecting an estimated relative reduction in cardiovascular morbidity and mortality of 24%.

The total number of patients randomized to achieve this goal, with a two-sided alpha of 0.05 and 80% power will be 1222. For this calculation we used dedicated software ('Power', dr. P.G.H. Mulder, Erasmus Medical Center, Rotterdam, the Netherlands). A study of this size has a statistical power of 80% at a two-sided alpha level of 0.05. The reason for using interim analysis [43] is that on average fewer patients are needed in the study when the expected difference in the primary outcome variable is real or when no difference can be expected anymore, therefore increasing efficiency. Sequential analyses are performed on survival outcome variables according to the double triangular test as described by Whitehead [44] and implemented in the computer program PEST version 4 [45]. The sequential (interim) analyses will be performed every three months by an independent data safety monitoring board (DSMB).

Statistical analysis

The analyses will be performed using the intention-to-treat principle. To assess whether intervention is related to a reduced risk of events we will use a chi-square analysis comparing the observed absolute risks across treatment groups. In addition, treatment efficacy will be assessed using a Cox regression model and expressed as a hazard ratio with corresponding 95 % confidence intervals. Since this is a randomized controlled trial, no adjustments will be made, although centers may be added. Associations will be considered significant at $p<0.05$. All statistical tests will be 2 sided. For statistical analyses we will use SPSS (SPSS for Windows, Chicago, Ill, SPSS Inc.).

Conclusions

Peripheral arterial disease is a common disease among elderly persons and is associated with a very high risk of cardiovascular events. In this study patients with peripheral arterial disease, but without cardiac symptoms, are randomized to an imaging arm consisting of multi-detector CT angiography of the coronary arteries and dobutamine stress MRI or to a control group in which case only a coronary calcium CT scan will be performed at baseline. In case of a positive finding in the imaging arm, patients are referred to a cardiologist who will take appropriate action. All participating patients will enter a 5-year follow-up for the occurrence of cardiovascular events. To the best of our knowledge GROUND is the first large trial designed to assess the value of multi-detector CT and MRI stress testing in reducing the morbidity and mortality of patients with peripheral arterial disease but yet without cardiac symptoms. At the time of writing this manuscript all centers are actively enrolling patients. The first patient enrolled in January 2005. The number of included patients is currently 228.

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Appendix

Participating Centers. The following hospitals in the Netherlands have agreed to participate in the GROUND-study: University Medical Center Groningen; University Medical Center Utrecht; St. Antonius Hospital Nieuwegein; Meander Medical Center, Amersfoort.

Executive Committee. The Executive Committee is responsible for the design of the GROUND study. It will coordinate and direct the study to ensure its overall success and decide on practical issues concerning the study. It will act upon

recommendations of the Data Safety and Monitoring Board regarding continuation of the study.
Members of the executive committee are:
Prof. W.P.Th.M. Mali, MD, PhD, University Medical Center Utrecht, responsible for the trial coordination
Prof. M. Oudkerk, MD, PhD, University Medical Center Groningen, responsible for the radiological coordination
Prof. F. Zijlstra, MD, PhD, University Hospital Groningen, responsible for the cardiological coordination
Dr. M.L. Bots, MD, PhD, University Medical Center Utrecht (Julius Center) responsible for the epidemiological coordination (project management, general data base management and statistical analyses)

Steering Committee. The steering committee consists of radiologists, cardiologists, epidemiologists, vascular surgeons and researchers of the participating centers. The Steering Committee will perform the actual imaging procedures of the study. Members will inform the Executive Committee on the progress of the study regularly. The steering committee and the executive committee will meet twice annually.
Members of the steering committee are: M. Prokop, MD, PhD; P.A. Doevendans, MD, PhD; A. Rutten, MD; A.M. de Vos, MD; F. Moll, MD, PhD; E.J. Vonken, MD, PhD; M.J.M. Cramer, MD, PhD; B.K. Velthuis, MD, PhD (University Medical Center Utrecht); H.J. van der Zaag, MD; R.A. Tio, MD, PhD; T.P. Willems, MD, PhD; P.M. van Ooijen, PhD; R. Vliegenthart, MD, PhD (University Medical Center Groningen); B. J. Rensing, MD, PhD; H.W. van Es, MD, PhD; H.D. van de Pavoordt, MD, PhD (St. Antonius Hospital Nieuwegein); A. Mosterd, MD, PhD; B.G. Heggelman, MD; R.A. Buiskool, MD; A.J. Mackaay, MD, PhD (Meander Medical Center Amersfoort)

Endpoint Committee. The Endpoint Committee will systematically evaluate suspected endpoints. Members are: F. Zijlstra, MD, PhD (cardiologist); B. Rensing, MD, PhD (cardiologist); A. Mosterd, MD, PhD (cardiologist); J. de Keyser, MD, PhD; W.J. Schonewille, MD (neurologist); T.W.M. Raaijmakers, MD, PhD (neurologist);

M.L. Bots, MD (epidemiologist), PhD; A. Rutten, MD (radiologist in training); A.M. de Vos, MD (cardiologist in training)

Data Safety and Monitoring Board. The data safety and monitoring board performs statistical analyses of un-blinded interim data and formulates recommendations for the Steering Committee on the continuation of the trial. The DSMB may also offer unsolicited recommendations on the continuation of the trial, for example after publication of results of similar trials. Every three months the chair of the DSMB will be provided an interim dataset to perform sequential analyses. When appropriate, given the results from the interim analysis, the chair will call for a meeting with the other DSMB members. Members of the Data Safety and Monitoring Board are: I. van der Tweel, PhD (statistician); M.J. M. Cramer, MD, PhD (cardiologist); H.J. van der Zaag, MD, PhD (epidemiologist); D.E. Grobbee, MD, PhD (epidemiologist)

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Chapter 3

High Prevalence of Treatable Severe Coronary Artery Disease: findings in a Randomized Controlled Trial in Cardiac Asymptomatic Peripheral Arterial Disease Patients

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submitted

ABSTRACT

Background: Patients with peripheral arterial disease (PAD) are at high risk for coronary artery disease (CAD). With non-invasive imaging modalities asymptomatic coronary atherosclerosis can be readily detected, and when found treated accordingly. We embarked on a randomized controlled trial in patients with symptomatic PAD but without previous cardiac disease to assess the value of non-invasive cardiac imaging using multidetector computed tomography (MDCT) and dobutamine stress magnetic resonance imaging (DSMR), as compared with standard care, in reducing cardiac events.

Methods and Results: PAD patients without objectified cardiac disease were randomized to undergo either extensive imaging or usual care. When with imaging a stenosis $\geq$ 50% in the left main coronary artery, or signs of inducible myocardial ischemia were found, patients were referred to a cardiologist for further diagnostic work-up and subsequent treatment. 231 Patients were randomized (n=115 in the imaging arm). In the imaging arm 22 patients (19%) had a stenosis of the left main coronary artery (or its equivalent). Of patients in whom a DSMR was performed (n=76), 2 had inducible ischemia. In total 24/115 (21%, 95% CI 14% - 29%) patients in the imaging group had evidence of severe CAD that required additional treatment on top of usual risk factor management.

Conclusions: Non-invasive cardiac imaging in cardiac asymptomatic PAD patients identifies a class I revascularisation indication in one fifth of patients. Our findings have a major impact on the management of these patients.

Introduction

Coronary artery disease (CAD) is the major cause of death in patients with peripheral arterial disease (PAD).[1-4] Furthermore, PAD is a common finding: in subjects older than 65 years, 21% have PAD. [5] Due to the generalized atherosclerosis in these patients, a considerable proportion of patients with symptomatic PAD, but free of symptomatic cardiac disease is likely to suffer from a coronary event. A recent review reported increased relative risks ranging up to 6.6.[3] According to the risk factor management guidelines, patients with symptomatic PAD should receive optimal risk factor treatment, i.e. aspirin, a statin and antihypertensive medication, if indicated and counseling with regard to physical exercise, healthy diet and cessation of smoking.[6]

With the availability of non-invasive imaging modalities nowadays, asymptomatic severe coronary atherosclerosis can be readily detected, and when found treated accordingly. Multi-detector computed tomography (MDCT) is a non-invasive modality that has been shown to be able to reliably detect significant CAD.[7;8] Without the use of contrast agents the amount of coronary calcium can be assessed, whereas the use of contrast allows for the assessment of luminal narrowing.

Dobutamine Stress Magnetic Resonance Imaging (DSMR) provides functional information on the myocardium under conditions of pharmacologically induced stress.[9-13] DSMR identifies left ventricular wall motion abnormalities indicative of myocardial ischemia, and can be used to assess viability in regions with rest wall motion abnormalities caused by prior myocardial infarctions.[9-13] As such, the information from the DSMR is complementary to that of contrast enhanced MDCT.

We therefore started a multi center randomized controlled trial with its main purpose to evaluate whether a cardiac imaging algorithm using non-invasive imaging techniques in cardiac asymptomatic PAD patients, followed by evidence based treatment in case of severe coronary pathology, can reduce the occurrence of cardiovascular events. Based on findings in the first 231 patients, as described here, the executive committee of the GROUND study decided in June 2007 that it is not defensible to proceed with the trial using a usual care treatment arm.

Methods

Patients

The study design has been described in detail elsewhere.[14] In brief, patients were recruited from the vascular surgery departments of the four participating centers (two university medical centers and two large general hospitals, all situated in the Netherlands). Patients were eligible if aged 50 years or older and diagnosed with lower extremity PAD (at least stage Fontaine II) by a vascular surgeon. Exclusion criteria were: physician diagnosed history of symptomatic CAD; heart rhythm other than sinus; unable to sustain a breath-hold for 25 seconds; asthma; contra-indications to DSMR examination; contra-indications to iodine contrast agent; severe arterial hypertension (>220/120 mmHg); significant aortic stenosis; unable to remain in supine position for at least 60 minutes; extreme obesity (BMI > 40 kg/m^2); renal insufficiency (serum creatinine level exceeding 140mmol/l); severe physical deterioration due to concomitant disease; language barrier; and contra-indications to dobutamine. Patients willing to participate were included after giving informed consent. The study was approved by the local medical ethical committees of the participating centers.

Baseline risk factors

At baseline, eligible patients completed a questionnaire on current medication use, risk factors and quality of life. In the clinic height, weight, blood pressure and systolic ankle pressure for calculation of the ankle-brachial index were measured. Total cholesterol, high density lipoproteins (HDL), triglycerides, creatinine, homocysteine, glucose and high sensitivity C - reactive protein were measured at the local laboratory. All patients were treated according to the Dutch guidelines for treatment of atherosclerotic PAD [15], which state that patients should receive aspirin, a statin and antihypertensive medication, if indicated. Patients also received counseling with regard to exercise, healthy diet and cessation of smoking.

Study design

Patients were randomized for either the imaging algorithm or the control group. In the control group only an unenhanced CT scan was performed to assess coronary calcium (figure 1). Patients randomized to the imaging arm underwent both an

unenhanced and an enhanced MDCT scan, allowing for the detection of coronary artery stenoses. In case of a significant stenosis (≥50% diameter reduction) of the left main coronary artery (LM) or its equivalent (the combination of significant disease in the proximal left anterior descending coronary artery (LAD) and the proximal left circumflex (LCx)), the patient was referred to a cardiologist for further diagnostic work-up and subsequent treatment at the cardiologist's discretion. The most recent guidelines state that it is a class I indication to perform coronary artery bypass grafting (CABG) in cardiac asymptomatic patients who have a significant stenosis in the LM or its equivalent.[16] All other patients underwent DSMR testing. Patients with signs of inducible ischemia during DSMR were also referred to a cardiologist. Furthermore, all patients referred to the cardiologist received appropriate anti-ischemic pharmacotherapy.[17] All patients are followed up and contacted biannually for the occurrence of any events.

MDCT imaging

All MDCT images were acquired at the local centers. In one center (UMCG) electron beam computed tomography (EBCT) (e-Speed, Imatron, San Francisco, USA) was used for the non-enhanced scans, whereas the MDCT scanner was used for the contrast-enhanced scans. That same center used a 64-MDCT scanner (Sensation 64, Siemens Medical Solutions, Forchheim, Germany) or a Dual Source Definition system by Siemens. The UMCU used a 64-MDCT scanner (Brilliance 64, Philips Medical Systems, Cleveland, OH, USA) and the other centers used a 16-MDCT scanner (Mx 8000 IDT 16, Philips Medical Systems, Cleveland, OH, USA or Somatom 16, Siemens Medical Solutions, Forchheim, Germany). For the patients randomized to the imaging arm of the study, calcium scoring was followed by a contrast enhanced retrospectively ECG-gated MDCT angiography. A beta-blocker (metoprolol) was administered to patients with a heart rate over 60 beats per minute.

The MDCT data were analyzed by the site investigator at the site, and a second central reading was performed by two experienced radiologists (MP and MO, both more than 15 years of experience). Calcium scoring was evaluated using an established analysis program (Advanced Workstation, GE, Chalford St. Giles, UK). The 16-segment tree from the American Heart Association (AHA) literature was used for segment definition. Each segment was evaluated for assessability, and reasons for

non-assessability were recorded. Reproducibility of the assessment of coronary calcium has been established earlier and was shown to be excellent.[18]

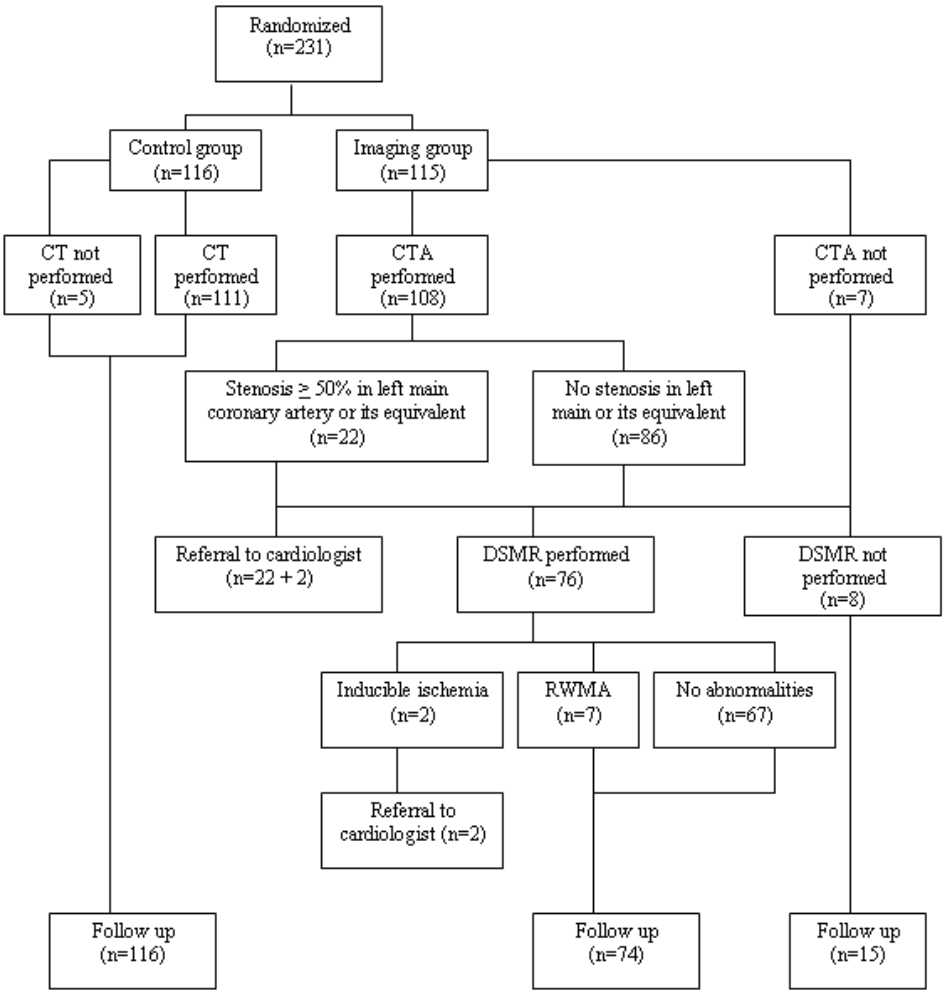

Figure 1. Numbers of patients who underwent randomization, imaging, and were available for follow up.

DSMR imaging

Patients randomized to the imaging group underwent DSMR within three weeks of the MDCT in one of the two university medical centers. In one center a 1.5 Tesla Sonata system by Siemens was used (UMCG), the other center used a 1.5 Tesla Philips Achieva system, release V1.56 (UMCU). All beta-blocking medication was stopped 4 days prior to the examination.[19] Dobutamine was infused intravenously and the doses were increased to 10, 20, 30 and 40 μg/kg/min with a six minutes' time

interval. In case of rest wall motion abnormalities (RWMA), infusion was started at 5 μg/kg/min. Image acquisition started three minutes after each dose increment. Criteria for ending the examination were (1) development of inducible wall motion abnormalities (IWMA) in more than 1 myocardial segment, (2) fall of systolic blood-pressure of >40 mmHg, (3) marked hypertension > 240/120 mmHg, (4) severe chest pain, (5) complex cardiac arrhythmia's, and (6) intolerable side effects of dobutamine. Both a radiologist and a cardiologist were present in the MR suite to monitor the condition of the patient and to directly evaluate the images.

The MR data were analyzed by the site investigator at the site and by a second central reader (more than 5 years of experience in DSMR testing). Image quality for every segment using the 16-segment model of the AHA was graded. Per segment wall motion was graded according to the guidelines of the American Society of Echocardiography.[20] A lack of increase in either wall motion or systolic wall thickening, a reduction of both or significant changes in the rotational pattern of left ventricular myocardium ('tethering') at increasing dobutamine doses were considered indicative of pathological findings. Myocardial ischemia was defined as IWMA in at least two segments at consecutive planes of the left ventricle. If WMAs were present at rest but improved during low-dose dobutamine stress, and worsened during peak-stress, this was also considered inducible myocardial ischemia. RWMAs that did not improve with low-dose dobutamine were not considered as inducible ischemia.

Inducible ischemia in cardiac asymptomatic patients with a significant lesion in one or two coronary arteries that are suitable for PCI is a class IIa indication for coronary revascularisation.[21] Whether this was actually done or not was left at the discretion of the treating cardiologist.

Statistical analysis

In the design phase of the study we anticipated to find left main stenoses or its equivalent in 8% of the patients, and inducible ischemia on DSMR in 22%, based on the scarcely available data in the literature. When we estimate the effect of revascularization therapy on patients with a left main stenosis or its equivalent to be a 70% reduction of events in 5 years, and the effect on patients with inducible ischemia a 40% reduction rate, the estimated sample size for our trial at the start was

1222 patients. For the current descriptive paper prevalence of abnormalities are being estimated and presented with 95% confidence limits.

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Between January 2005 and March 2007, 231 PAD patients free of a history of cardiac symptoms were included, of whom 115 were randomized to the imaging group, and 116 to the control group. General characteristics for both groups are presented in Table 1. Characteristics were well balanced between treatment groups. Risk factor levels were high in this patient group, diabetes mellitus was common, and almost all participants smoked. Left ankle-brachial index (ABI) in both groups was 0.82 (SD 0.22), but the right ABI was higher in the imaging group, 0.90 (SD 0.25) versus 0.83 (SD 0.24).

In 12 patients (5 in the control group) the MDCT scan was not performed because of various reasons (no show, condition deteriorated, logistic reasons, withdrawal of consent), leaving 219 CT scans for analysis (Figure 1). From 111 patients in the control group the calcium score was calculated, and in the imaging-with-treatment arm, the calcium score was calculated and a contrast enhanced scan was made of 108 patients. Of these, 5 scans were of non-diagnostic quality for assessment of the LM or equivalent artery (n=3) or other segments (n=2) due to arrhythmia (n=3) or breathing artifacts (n=2). These scans were considered negative for a stenosis in the LM or its equivalent. Fifty-three (46%) of the 115 patients who were randomized to the imaging arm had at least one significant stenosis anywhere. Stenoses were most prevalent in the proximal (n=27; 23%) and mid (n=21; 18%) LAD. One-vessel disease was present in 21(18%) patients, two-vessel disease in 23 (20%) and three-vessel disease in 16 (14%).

Twenty-two (19%) patients had a stenosis in the LM (n=13), and/or its equivalent (n=14). These patients were referred to a cardiologist for diagnostic work-up and subsequent therapy (Table 2). In all but two of these patients a CAG was performed, which confirmed the presence of a severe stenosis that required invasive intervention in 16 (14%) patients. Two patients were additionally referred to the cardiologist without the presence of a stenosis in the LM or its equivalent but for

diagnostic work up because of other major abnormal cardiovascular findings at MDCT (aberrant right coronary artery, penetrating atherosclerotic ulcer in the thoracic aorta). In the patient referred for an aberrant course of the RCA other tests than CAG were performed which ruled out ischemia, the other patient underwent surgery. Double reading of the CTA's provided identical results.

Eight patients subsequently underwent a coronary artery bypass grafting procedure (CABG), 3 had a percutaneous coronary intervention (PCI) and 5 patients were not treated invasively despite an indication for revascularization. In one patient the coronary anatomy prohibited revascularization therapy, and in the other 4 patients the cardiologist decided not to intervene in the absence of any cardiac symptoms, but to intensify pharmacotherapy. There were no peri-procedural complications of the revascularizations.

Of the 84 patients who should have undergone a DSMR (Figure 1), 8 patients were not scanned due to various reasons (patient refused, contraindications to MRI that had not been identified earlier). Median time between MDCT and DSMR was 17 days. In 7 patients (6% of patients randomized to imaging group) RWMAs were found, indicating previous (silent) myocardial infarction, and in 2 patients (1.7%) inducible ischemia was observed. In another 3 patients inducible ischemia was observed in only one segment. The patients with inducible ischemia were referred to the cardiologist, who performed a CAG in both, which confirmed significant stenoses and PCI was performed in 1 patient, the other was treated with intensive pharmacotherapy. Thus, in total 24/115 (21%, 95% CI 14% - 29%) patients in the imaging group had evidence of severe coronary artery disease (CAD) that required additional treatment on top of usual risk factor management. All patients with significant CAD received appropriate anti-ischemic pharmacotherapy.

Median Agatston calcium score of all 219 patients was 276 (range 0 to 5135). The distribution of patients in scores according to Rumberger et al.[22] showed that most patients (n=87; 40%) had a score higher than 400, and 17 patients (8%) had no detectable calcium in their coronary arteries (Table 1).

In addition to the patients with a cardiac diagnosis who were referred to the cardiologist, three patients were referred to other specialists because of suspicious lesions. One was diagnosed with a non-Hodgkin's lymphoma, one had a T1N0M0

lung carcinoma which was then surgically radically removed, and the third patient had pulmonary lesions on MRI that were diagnosed as residual abnormalities.

Table 1. Comparison of baseline risk factors in randomization groups (n=231) and calcium scores (n=219)

	Imaging group (n=115)	Control group (n=116)
Age	62 (7.3)	62 (7.2)
Male, n (%)	91 (80%)	82 (71%)
Ever diagnosed with hypertension	45%	48%
Systolic blood pressure (mm Hg)	147 (23)	148 (23)
Diastolic blood pressure (mm Hg)	83 (10)	83 (10)
Ever diagnosed with hypercholesterolemia	61%	63%
Total cholesterol (mmol/l)	4.9 (1.2)	4.8 (1.1)
HDL-cholesterol (mmol/l)	1.4 (0.4)	1.4 (0.4)
Triglycerides (mmol/l)	1.8 (1.0)	1.8 (1.2)
Ever diagnosed with diabetes	20%	22%
Glucose (non-fasting) (mmol/l)	5.8 (1.7)	5.8 (1.7)
Creatinine (mmol/l)	87.6 (15.6)	83.8 (15.1)
Homocysteine (umol/l)	12.1 (4.3)	12.0 (4.4)
CRP, median (range) (mg/l)	2.1 (0.2 to 21.0)	2.4 (0.2 to 23.6)
Current smoker	51%	49%
Current or former smoker	96%	98%
Calcium scoring, median (range)	N=108	N=111
Agatston score	259 (0 to 5135)	284 (0 to 3174)
Mass score	52 (0 to 3362)	70 (0 to 1863)
Volume score	256 (0 to 6668)	241 (0 to 2944)
Calcium scores according to Rumberger, n (%)		
0 to 1	9 (8%)	8 (7%)
1 to 10	9 (8%)	10 (9%)
11 to 100	21 (19%)	16 (15%)
101 to 400	28 (25%)	31 (29%)
> 401	44 (40%)	43 (40%)

CRP= high sensitivity C-reactive protein

Discussion

In PAD patients without a history of cardiac symptoms, we observed a strikingly high prevalence of severe coronary heart disease (i.e., 19%) with a class I indication for revascularization on CT and another 2% with inducible ischemia.

Patients with PAD are at high risk for CVD mortality and morbidity, perhaps even more than patients with a prior myocardial infarction.[3;23] Presence of coronary heart disease among those with premature PAD (age of onset < 45 years) but free from coronary symptoms has been reported to be 10%. [24] In a study among 57 patients with a renal artery stenosis and free from cardiac symptoms, a significant stenosis was found in 17% of the patients. [25] A series of 302 patients with an aneurism of the aorta revealed correctable CAD on angiogram in 19% of those in whom CAD was not suspected. [26]

In 1984, Hertzer et al. reported results of a retrospective database of coronary angiography (CAG) in 1000 patients scheduled for vascular surgery because of lower extremity vascular disease, cerebrovascular disease or abdominal aortic aneurysm.[27] They found severe coronary abnormalities in 15% of patients. This study is however outdated, and serious selection bias occurred due to the invasive procedure that served as the diagnostic tool and included both cardiac symptomatic and asymptomatic patients. The reported rate further included patients with single vessel disease and the number of patients with significant left main disease, comparable to our definition of severe CAD, was only 4%.

A recent meta-analysis indicated that the prevalence of both PAD and CAD in one patient differs tremendously in literature, depending on the sensitivity of the diagnostic technique used to detect CAD.[3] It is well known that patients with PAD, also without cardiac symptoms, are at increased risk of suffering from a coronary event.[27] This has been attributed to the systemic nature of atherosclerosis development. Yet information on the prevalence of coronary atherosclerosis in this cardiac asymptomatic patient population is limited and the prevalence of LM (or equivalent) stenoses has never been described to be this high. Our study population consisted of those patients, who were willing to participate and obviously were alive. Thus, the reported prevalence of severe coronary disease is likely an underestimate because of the occurrence of death before enrolment in subjects with fast-evolving atherosclerotic disease.

The GROUND study was designed to study whether imaging and subsequent treatment results in a reduction of cardiac morbidity and mortality. Yet, based on the high prevalence of stenoses with a class I revascularization indication, the executive committee found it indefensible to continue the usual care arm of the trial. At the planning of the study, we had anticipated a prevalence of LM stenosis or its equivalent of 8%. We found a prevalence of 19% (95% CI: 12% to 27%) on CT.

Some limitations of our study have to be addressed. First, we have a relatively small sample which limits the precision of our findings. Second, 20 scans in total (both MDCT and DSMR scans) were not performed due to logistic and patient related reasons. Although this dilutes our rates (we present intention-to-diagnose rates), we do not think that this has lead to a considerable bias, since reasons for the violations were not patient-related, but more technically and logistically. Also, it resembles daily practice in large general hospitals well, which increases the generalisability of our findings.

The COURAGE trial [17] showed us that invasive therapy in selected patients with stable coronary artery disease does not reduce mortality as compared to optimal medical therapy. However, patients with LM disease were not included in the study.

Although we found more CAD than anticipated, the number of positive DSMR scans was a lot less than expected. We have no reason to believe that the quality of the scans was not good, so after careful identification of patients with anatomical evidence of severe CAD, disease limited to a singe and/or distal coronary artery does not often lead to objective evidence of inducible myocardial ischemia.

In summary we present a very high rate of co-occurring CAD in cardiac asymptomatic PAD patients, with 19% having a class I revascularization indication. [21] Given the high prevalence of structural abnormalities in the coronary arteries found in cardiac asymptomatic vascular disease and diabetic patients, the associated worse prognosis, the presence of a class I indication for revascularization, and the availability of a non-invasive imaging technique capable of assessment of structural abnormalities, we believe a comprehensive research program is needed to provide the evidence on the desirability of screening for severe coronary stenoses using cardiac CT with subsequent evidence based treatment in cardiac asymptomatic patients with either non-cardiac vascular disease or diabetes mellitus in order to reduce vascular events in this high risk patient group.

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Chapter 4

The prevalence and localization of coronary atherosclerosis in cardiac asymptomatic patients with peripheral arterial disease

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Abstract

Background and objective: In patients with peripheral arterial disease (PAD) mortality due to coronary artery disease is known to be high. However, the prevalence and localization of atherosclerotic plaques in the coronary arteries in this patient group is unknown. In the GROUND study cardiac asymptomatic patients with PAD were screened for coronary artery disease (CAD) with cardiac CT angiography (cCTA). Here we report on the prevalence and localization of coronary plaque in cardiac asymptomatic patients with PAD.

Methods: In 108 PAD patients without anginal symptoms cCTA was performed. All scans were analyzed for coronary artery assessability, stenosis grade (% diameter reduction) and plaques composition (non-calcified, mixed, calcified) using a 16-segment model.

Results: Mean age was 62±7years and 91 (80%) participants were men. In 3 participants none of the proximal coronary arteries were assessable (2.8%). In 9 persons (8.3%) the mid coronary arteries and in 9 persons the distal coronary arteries were not assessable. Significant stenoses were present in 53 of the 108 (49.1%) patients. In 46 (42.6%) patients a stenosis was located in the proximal or mid coronary arteries. In 98 (90.7%) patients atherosclerotic plaque of any type (calcified, mixed or non-calcified) was present. 56 (51.9%) had a plaque in the left main coronary artery. Non-calcified plaques were present in 51 (47.2%), calcified plaques in 84 (77.8) and mixed plaques in 70 (64.8) of patients. Of all 22 (20.4%) patients without any calcified or mixed plaque, 2 (1.9%) had a significant stenosis.

Conclusion: In a patient group with PAD, who are cardiac asymptomatic, the prevalence of coronary artery stenoses and atherosclerotic plaques is very high. Most plaques are localized in the LAD and in the proximal segments of the coronary artery tree.

Background

Of all patients with established atherosclerotic disease those with lower-extremity peripheral arterial disease (PAD) have the worst prognosis [1]. Despite the relatively benign prognosis for the affected limb, symptoms of intermittent claudication should be regarded as a sign of systemic vascular disease. Coronary artery disease (CAD) is the important other manifestation of this disease and forms a major cause of death in these patients[2;3]. Irrespective of whether symptoms are present, PAD patients have an increased risk of myocardial infarction and are 6 times more likely to die within10 years than patients without PAD[4;5].

PAD is a common disease[4;6;7], especially in elderly persons[8], and its related health care costs are not to be underestimated [9]. The prevalence PAD in Europe and North America is approximately 27 million people[10], 16% of the population of 55 and older have PAD, 10.5 million people are symptomatic and the majority, 16,5 million are asymptomatic. Reports from the Framingham Heart Study suggest that the prevalence of PAD has increased over the past 30 years [11;12].

In 1984, Hertzer et al. performed coronary angiograms in 166 patients with lower extremity PAD, who were not suspected of CAD, and revealed the presence of CAD in 86% of these cardiac complaint free PAD patients[13]. In the general population 2.8% of asymptomatic people show angiographic signs of atherosclerosis[14].

The risk and discomfort of undergoing conventional coronary angiography is not to be neglected[15-17]. Today, coronary computed tomography angiography (cCTA) offers a much less invasive screening tool. Modern scanners show high sensitivities in detecting both obstructive[18-20] and non-obstructive[21] coronary stenoses and screening asymptomatic patients for the presence of CAD appears feasible [22;23]. As opposed to conventional angiography, a distinction can be made between non-calcified, mixed and calcified coronary plaques [23-26]. The importance of detecting non-calcified plaques lies in the fact that rupture prone plaques are often non-calcified plaques that tend to preserve a normal lumen due to expansive remodeling and are thus not detectable with conventional angiography[27-29]. To the best of our knowledge, this is the first cCTA study to present the prevalence and localization of coronary atherosclerosis in cardiac asymptomatic patients with PAD.

Methods

For the current study we used baseline data from 108 patients who were randomized to the imaging arm of the "Ground study" and subsequently underwent cCTA. The GROUND study was a multicenter randomized controlled clinical trial with a 5-year follow up period, which was performed in 4 hospitals in the Netherlands. The methods of the GROUND study have been described in an extensive manner elsewhere[30]. The study protocol was approved by the Institutional Review Board of the University Medical Centers Utrecht and Groningen, Meander Medical Center Amersfoort and the Antonius Hospital Nieuwegein, the Netherlands. All participants gave their written informed consent.

Patient selection

Patients with PAD and without a history of symptomatic cardiac disease were recruited from the vascular surgery departments of the participating centers. At the University Medical Center Utrecht cardiac asymptomatic PAD patients were identified from the SMART cohort study[31]. Patients were eligible if 50 years or older and having lower extremity PAD, at least stage Fontaine II, as diagnosed by the vascular surgeon. Patients were considered not eligible for the study if they met one of the following exclusion criteria: physician diagnosed history of symptomatic cardiac disease; cardiac rhythm other than sinus; unable to sustain a breath-hold for 25 seconds; asthma; contra-indications to MRI examination; contra-indications to iodine contrast agent; severe arterial hypertension (>220/120 mmHg); significant aortic stenosis; unable to remain in supine position for at least 60 minutes; extreme obesity (BMI > 40 kg/m^2); renal insufficiency (serum creatinine level exceeding 140mmol/l); severe physical deterioration due to concomitant disease; language barrier; and contra-indications to dobutamine.

Risk factor assessment

Participants completed a questionnaire on current medication use, risk factors and quality of life. In the outpatient clinic height, weight, blood pressure and ankle pressure for calculation of the ankle-brachial index were measured. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Total cholesterol, high density lipoproteins (HDL), triglycerides, creatinine, homocysteine, glucose and high

sensitivity c-reactive protein were measured at the local laboratory. To prevent further progression of their present cardiovascular disease all patients were treated according to the Dutch guidelines for treatment of atherosclerotic peripheral arterial disease[32]. These guidelines state that these patients should receive aspirin, a statin and antihypertensive medication, if indicated. Patients received also proper advice regarding exercise, healthy diet and cessation of smoking.

Coronary CT Angiography

Half of the patients were randomized to the imaging arm of the trial (n=115). Of these participants 7 did not undergo cCTa due to various reasons (condition deteriorated, logistic reasons, withdrawal of consent). All participating centers are experienced in making cCTA scans. The University Medical Center Utrecht and the University Medical Center Groningen used a 64-slice CT scanner (Brilliance 64, Philips Medical Systems, Cleveland, OH, USA and Sensation 64, Siemens Medical Solutions, Forchheim, Germany, respectively) and the Antonius Hospital Nieuwegein and the Meander Medical Center Amersfoort both used a 16-slice CT scanner (Mx 8000 IDT 16, Philips Medical Systems, Cleveland, OH, USA and Somatom 16, Siemens Medical Solutions, Forchheim, Germany, respectively). For the patients randomized to the imaging arm of the study, calcium scoring was followed by contrast enhanced retrospectively ECG-gated CT angiography. Patients with a heart rate > 60 bpm received a beta-blocker (intravenous metoprolol, 5 to 20 mg), while blood pressure was monitored. The cardiac phase with the least amount of motion was identified. This phase was then loaded into the appropriate application of the cardiac software program. Depending on the coronary morphology and quality of the scan several post processing techniques were applied to assess the coronary arteries. The dataset was evaluated in terms of assessability, stenoses and plaques. The AHA classification of coronary segments was used [33]. 'Proximal segments' are segments 1, 5, 6 and 11. 'Mid segments' are segments 2, 7, and 13. Segments 3, 4, 8, 9, 10, 12, 14, 15 and 16 are considered 'distal'. Each segment was scored for assessability on a 5-point scale (1=non-diagnostic to 5=excellent). Segments with more than 3 points were used in the analyses. Any luminal narrowing greater than 30% was visualized from the curved MPR and quantified according to a 4-point scale: (1) 30-50%, (2) 50-70%, (3) 70-99% and (4) 100%. Coronary atherosclerotic plaque was classified as non-calcified (no

visible calcification and lower density than the contrast-enhanced vessel lumen), calcified (plaques with high density) or mixed (plaques with both calcified and non-calcified parts).

Statistical analysis

Continuous data are presented as mean ± standard deviation (SD) and compared using the Student *t* test. Categorical data were expressed as percent frequencies, and differences between proportions were compared using the chi-square test. For all tests, a p value <0.05 (two-sided) was considered significant. All analyses were performed using SPSS-12.0.2 statistical software (SPSS Inc., Chicago, Illinois).

Results

Clinical characteristics of the 108 GROUND study participants who underwent a cCTA are shown in table 1. The mean age was 62±7years and 91 (80%) participants were men. In 3 participants none of the segments of the proximal coronary arteries were assessable (2.8%). In 9 persons (8.3%) the mid coronary arteries and in another 9 persons no segment of the distal coronary arteries was assessable.

Significant stenoses (≥50% luminal diameter reduction) were present in 53 of the 108 (49.1%) patients who underwent cCTA (Table 2). Most patients had a stenosis in the left anterior descending coronary artery (LAD). In 46 (42.6%) patients a significant stenosis was located in the proximal or mid coronary arteries.

In 98 patients (90.7%) at least one atherosclerotic plaque of any type (calcified, mixed or non-calcified) was present. Most participants, 88 (81.5%) had a plaque in the LAD and 56 (51.9%) had a plaque in the left main coronary artery (LM). Non-calcified plaques were present in 51 (47.2%), calcified plaques in 84 (77.8) and mixed plaques in 70 (64.8) of patients. The distribution of plaque types was not different between men and women. Atherosclerotic plaques of any type were more prevalent among men than women (95% vs 81%; p=0.026). Calcified plaques were also more prevalent among men than among women (83,5% vs 61.9%; p=0.029). Of all 22 (20.4%) patients without any calcified or mixed plaque, 2 (1.9%) had a significant stenosis: one in the proximal RCA and one in the distal LAD.

Table 1. Clinical Characteristics of the 108 participants who underwent cCTA.

	patients who underwent cCTA (n=108)
Age	62 (7.1)
Weight	82 (12.2)
BMI	26.4 (3.5)
Male, n (%)	91 (80%)
Ever diagnosed with hypertension	45%
Systolic blood pressure (mm Hg)	146 (22)
Diastolic blood pressure (mm Hg)	83 (10)
Ever diagnosed with hypercholesterolemia	61%
Total cholesterol (mmol/l)	4.9 (1.2)
HDL-cholesterol (mmol/l)	1.4 (0.4)
Triglycerides (mmol/l)	1.8 (1.0)
Ever diagnosed with diabetes	20%
ABI left	0.83 (0.22)
ABI right	0.91 (0.25)
Glucose (non-fasting) (mmol/l)	5.8 (1.8)
Creatinine (mmol/l)	87.6 (15.6)
Homocysteine (umol/l)	12.1 (4.3)
Current smoker	51%
Current or former smoker	96%

HDL denotes high density lipoproteins; ABI denotes ankle-brachial index; BMI denotes body mass index

Table 2. The number of persons with signs of coronary atherosclerosis.

	total	RCA	LAD	LCX	LM	proximal	mid	proximal or mid	distal and side
Characteristic, n (%)	**n=108**								
at least one significant stenosis ≥50%	53 (49.1)	27 (25.0)	44 (40.7)	22 (20.4)	13 (12.0)	36 (33.3)	32 (29,6)	46 (42.6)	27 (25.0)
at least one plaque of any type	98 (90.7)	69 (63.9)	88 (81.5)	56 (51.9)	56 (51.9)	93 (86.1)	73 (67.6)	95 (88.0)	62 (57.4)
at least one non-calcified plaque	51 (47.2)	26 (24.1)	29 (26.9)	19 (17.6)	19 (17.6)	37 (34.3)	25 (23.1)	49 (45.4)	21 (19.4)
at least one calcified plaque	84 (77.8)	55 (50.9)	58 (53.7)	34 (31.5)	19 (17.6)	70 (64.8)	51 (47.2)	78 (72.2)	46 (42.6)
at least one mixed plaque	70 (64.8)	30 (27.8)	52 (48.1)	19 (17.6)	25 (23.2)	60 (55.7)	40 (37.0)	68 (63.0)	24 (22.2)

RCA right coronary artery; LAD left anterior descending coronary artery; LCX circumflex coronary artery; LM left main coronary artery. 'proximal' denotes proximal coronary arteries: segments 1, 5, 6 and 11; 'mid' denotes mid coronary arteries: segments 2, 7 and 13. 'distal and side' denotes distal coronary arteries: segments 3, 4, 8, 9, 10, 12, 14, 15 and 16 according to the American Heart Association definition

Discussion

In this study we showed a considerable prevalence of coronary atherosclerosis in cardiac asymptomatic patients with lower-extremity PAD. More than 90% of our study population shows signs of occult coronary atherosclerosis and almost half had a considerable coronary stenosis. The numbers we present are likely to be an underestimation, because unassessable segments were not counted.

The high prevalence of calcified, mixed and non-calcified plaques forms no indication for invasive measures in this asymptomatic patient group according to the current guidelines [34]. In asymptomatic patients only significant stenosis of the left main coronary artery or its equivalent or three-vessel-disease form an indication for revascularization. The prognostic implications of these plaques need to be prospectively evaluated to determine the value of our findings for future cardiovascular events in order to balance whether intervention might be needed.

The general public and those suffering from PAD are largely unaware that PAD is a powerful risk marker for heart attack, stroke and death[35]. PAD in the absence of known CAD relates to cardiovascular events more strongly than CAD in the absence of PAD in the Heart Protection Study (30.5% versus 22.5% event rate, placebo group)[36]. The large coronary plaque burden in our study underscores these findings by showing the pathophysiologic basis of the increased risk of cardiovascular events in this patient group.

Having no calcified coronary plaques does not rule out significant CAD [37]. In 2 patients without calcified or mixed plaque we even found a significant coronary stenosis. This finding is in concordance with the significant LM stenosis in a patient with a calcium score below 10 (chapter 4) in the same population.

The presence of coronary stenoses in cardiac asymptomatic PAD patients has been described before with conventional coronary angiography (CAG) [13]. Several studies have shown that diagnostic CAG has an average morbidity of 2% and a mortality of approximately 0.1% [17;38;39]. For screening purposes non-invasive imaging would be more suitable. The rapid development of multi-detector computed tomography has made it possible to image the heart and its coronary arteries in a non-invasive way. It is much faster than older CT scanners and images are obtained with sub millimeter spatial resolution and high temporal resolution. As a result of simultaneous recording of an electrocardiogram (ECG) signal, several image

reconstructions are possible in different phases of the heart cycle. [19;40] Not only the costs and risk of complications are lower with cCTA than with CAG, this technique also has the advantage of vessel wall visualization. Both the composition of the plaque and its impact on the vessel lumen can be detected. A distinction can be made between lipid, fibrous and calcified coronary plaques [26;41;42].
In recent years it has become clear that plaque composition may be a better risk-predictor for acute coronary events than stenosis grade. Rupture of so called vulnerable plaques accounts for approximately 70% of sudden coronary deaths. Although the average absolute risk of severely stenotic plaques may be higher than the average absolute risk of mildly stenotic plaques, the number of plaques with mild stenoses overwhelmingly exceeds the number of plaques with severe stenoses[43]. Acute coronary syndromes are caused by plaque erosion or rupture and often these plaques are noncalcified[43] and form no luminal narowing[29]. The prognostic value of these non stenotic plaques on cCTA has to be established in large follow up studies. Detection of these plaques with cCTA could potentially identify patients at even higher risk. Assessment of cardiac atherosclerotic abnormalities using non-invasive techniques followed by appropriate treatment may help to improve survival in PAD patients without anginal complaints.

Limitations of the present study should be considered. First, presented coronary stenoses were not confirmed with conventional CAG. Although cCTA has proven to be an accurate non invasive imaging tool, it remains difficult to visualize plaques in the distal and mid sections of the coronary tree[44;45]. Radiation exposure remains an important issue in cCTA and should not be underestimated [46], especially in women, although rapid development in software, hardware and scan protocols will reduce radiation dose in the future.

In conclusion, we present a high prevalence of coronary stenoses and plaques in patients with lower-extremity PAD, who are without cardiac symptoms. These atherosclerotic plaques are mostly located in the LAD and the proximal coronary arteries. cCTA seems a valuable method for detecting these stenoses and plaques.

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Chapter 5

The utility of coronary calcium scoring in cardiac asymptomatic patients with peripheral arterial disease

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Abstract

Introduction: Patients with peripheral arterial disease (PAD) are known to have a high risk for cardiac morbidity and mortality. These patients are eligible for secondary prevention of cardiac disease. We determined whether on top of risk factors coronary calcification, a risk indicator for cardiac disease, can help predict the occurrence of significant left main (LM) stenosis or equivalent, eligible for surgery, in this high-risk group.

Methods: 219 cardiac asymptomatic patients with peripheral arterial disease underwent risk factor assessment and non-contrast-enhanced computed tomography (CT) scanning of the heart for calcium scoring. 108 of these patients were randomized to also undergo contrast-enhanced coronary CT angiography (cCTA) to determine the presence of a LM (equivalent) stenosis. If positive, invasive coronary angiography was performed and if indicated, surgery followed. Relations between risk factors and Agatston calcium score were determined. We used logistic regression modeling to evaluate the role of risk factors and Agatston score for the prediction of the presence of a significant LM stenosis, eligible for surgery.

Results: Median Agatston score was 276 (range 0-5135). Only an increase in age was significantly related to Agatston score ($p<0.05$). Twenty-two of 108 patients had a significant LM (equivalent) stenosis on cCTA. Eight of these twenty-two patients were eligible for surgery. Diabetes and score >400 were independent predictors of significant LM stenosis, with a 6.1 and 12.4-fold increased risk, respectively. These two factors were included in a multivariable logistic regression model for the presence of a significant LM stenosis, eligible for surgery (ROC-area 0.82; 95%CI 0.65-0.98).

Conclusion: Calcium scores and diabetic status may help in predicting the occurrence of significant coronary artery disease, eligible for surgery, in cardiac asymptomatic PAD patients.

Introduction

Patients with peripheral arterial disease (PAD) are at a high risk to have or develop concomitant coronary artery disease since both peripheral arterial disease and coronary artery disease are caused by atherosclerosis[1-4]. Cardiac morbidity and mortality are increased in PAD patients compared to in the general population [1;5;6]. Their risk for future cardiac events may be considered equivalent to the risk of individuals with a previous cardiac event[7;8]. This PAD patient group may therefore be eligible for screening for significant cardiac disease, e.g. left main coronary artery stenosis, and treatment in a cardiac asymptomatic stage.

Studies in large asymptomatic populations show that quantification of the amount of coronary calcification can aid in determining the risk for future cardiac morbidity and mortality[9-13]. PAD patients, however, already are expected to be in the highest calcium scoring risk group due to the presence of atherosclerosis in their peripheral arteries. It is unknown if calcium scoring can be used in a high-risk cardiac asymptomatic peripheral arterial disease patient group to further differentiate risk.

We studied the occurrence of coronary calcification in a high-risk group of cardiac asymptomatic patients with PAD in relation to conventional cardiovascular risk factors and we determined whether the amount of coronary calcification on top of risk factors may be of help in predicting the occurrence of a significant left main or left main equivalent coronary artery stenosis, eligible for surgery in an asymptomatic stage[14], in this high-risk group.

Methods

Subjects are part of an ongoing large randomized multi-center trial, the GROUND study, into the effects on vascular events of screening for and treatment of an asymptomatic stage of significant cardiac disease (registered at ClinicalTrials.gov as NCT00189111)[15]. The study was approved by the (local) Ethics Committee and patients signed informed consent after obtaining written and oral information. Subsequently they were randomized to either the first group that would undergo an elaborate non-invasive cardiac imaging algorithm including CT calcium scoring, coronary CT angiography and dobutamine stress MRI (DSMR) with subsequent

evidence based treatment depending on the imaging results (intervention group) or the group that would only undergo CT calcium scoring (control group). Regardless of the results of randomization, risk factor assessment was performed and patients received treatment advice (medical and lifestyle) according to the current Dutch standards for the treatment of patients with peripheral arterial disease[16]. All patients entered a 5-year follow-up after inclusion. Only baseline risk factor data and CT data are included in the following analysis.

Patient selection

Subjects were recruited between January 2005 and February 2007. Patients, aged 50 years or over, diagnosed with intermittent claudication by a vascular surgeon were eligible for this trial. Exclusion criteria were a history of symptomatic cardiac disease, arrhythmia, inability to sustain breath-hold for 25 seconds, asthma, contra-indications to MRI examination or dobutamine, contrast medium allergy, renal insufficiency, severe arterial hypertension (>220/120 mmHg), extreme obesity (BMI>40 kg/m2), severe co-morbidity and/or a language barrier.

Risk factor screening

At baseline all trial participants completed a comprehensive questionnaire on medical history, medication use and quality of life. Height, weight and blood pressure at each arm were measured. Body mass index (BMI) was calculated as weight divided by height squared. Blood samples were taken to measure levels of total cholesterol and of creatinine to determine presence of hypercholesterolemia and to obtain a measure of renal function, respectively. Smoking status was subdivided in never and ever (i.e. both quit and current). No difference was made between quit and current, since most patients quit smoking shortly before inclusion. Hypertension was defined as a systolic blood pressure equal or higher than 140 mmHg and/or a diastolic blood pressure equal or higher than 90 mmHg and/or use of antihypertensive medication. Diabetes mellitus was determined by self-reporting and/or use of glucose lowering medication. Hypercholesterolemia was also determined by self-reporting and/or as a total cholesterol higher than 6.5 mmol/l. Cholesterol lowering medication use was not taken into account since these are prescribed preventively.

CT calcium scoring acquisition and evaluation

CT calcium scoring was performed on either a 16- or a 64-detector-row CT scanner (Mx8000 IDT 16 or Brilliance 64, Philips Medical Systems, Cleveland, OH, USA; Somatom 16, Sensation 64 or Dual Source Definition, Siemens Medical Solutions, Forchheim, Germany) or an EBT scanner (only CT calcium scoring scans; e-Speed, Imatron, San Francisco, USA) depending on the center of inclusion. Each center used its own optimal imaging protocol for the scan acquisition. The calcium scoring scan was loaded in an analysis program to determine the calcium score. A trained observer identified coronary calcifications in each coronary artery to obtain a total Agatston score[17].

Coronary CT angiography acquisition and evaluation

The subjects in the screening group underwent contrast-enhanced cCTA after the calcium scoring scan acquisition. The cCTA was performed on either a 16- or a 64-detector-row CT scanner (Table 1). Subjects received beta-blockers in case of a heart rate above 60 bpm. Contrast medium volume and infusion rate during cCTA depended on patient weight and scan duration. Image data sets were reconstructed at several time points of the RR-interval and the most-motion free data set was selected to evaluate the left main, proximal left anterior descending and proximal left circumflex coronary artery for plaques and stenoses (segments 5, 6 and 11, respectively, according to the 15-segment tree of the AHA). Each segment was scored for assessability on a 5-point scale (1=non-diagnostic to 5=excellent). The reason for non-assessability was also recorded. Luminal narrowing was visually determined and subdivided in 5 categories: 0-30%, 30-50%, 50-70%, 70-99% and 100%. Plaque types in each segment were recorded as soft, calcified or mixed (both soft and calcified components). Patients with a significant (>50%) left main stenosis or a significant left main equivalent stenosis (>50% stenosis of both proximal LAD and LCx coronary artery) were referred to a cardiologist.

Invasive coronary angiography (CAG)

All patients referred to a cardiologist underwent CAG unless contra-indicated. All studies were performed using digital equipment. Multiple projections were recorded

for each vessel using standard orientations. Cine-fluoroscopy images were analyzed for significant left main (equivalent) stenosis by the cardiologist performing the CAG. In case a significant (equivalent) left main stenosis was detected on CAG coronary artery bypass graft (CABG) surgery was performed, if deemed feasible.

Data analysis

Continuous variables are presented as mean with standard deviation. Categorical variables are given as percentages in each category. Calcium scores were subdivided based on the groups defined by Rumberger (0, 1-10, 11-100, 101-400, >400). Differences in cardiovascular risk factors were determined between calcium score groups with an ANOVA test for continuous data and a Kruskal-Wallis test in case of categorical variables. Univariable logistic regression was performed to determine the association between cardiovascular risk factors or calcium score groups and presence of a significant (equivalent) left main stenosis on cCTA, confirmed with CAG in the intervention group. The odds ratios were used as measures of association. All variables with a $p<0.157$ in univariable logistic regression were used for multivariable logistic regression. The prognostic ability of the models, i.e. to discriminate between patients with and without significant disease, was estimated by measuring the area under the receiver operating characteristic (ROC) curves[18].

Results

231 subjects were randomized to either the intervention (n=115) or the control group (n=116) (Figure 1). A calcium scoring scan was successfully performed in 219 subjects out of the 231 randomized subjects (108 intervention group, 111 control group). The other 12 subjects did not undergo CT imaging because of various reasons (personal reasons, sudden deteriorating physical condition or logistic reasons). In the 219 subjects who underwent calcium scoring mean age was 62.4 (±7.1) years and 164 patients were male (76%). Patient characteristics with regard to risk factors are shown in Table 1.

Table 1. Patient characteristics at baseline

	Overall n=219	
Age (years)	62.4	(7.1)
Male sex	75.8%	
BMI (kg/m^2)	26.3	(3.6)
Systolic blood pressure (SBP) (mmHg)	146.6	(22.4)
Diastolic blood pressure (DBP) (mmHg)	82.2	(9.8)
Hypertension (SBP>140mmHg, DBP>90mmHg, medication)	69.0%	
Total cholesterol (mmol/l)	4.84	(1.16)
Total cholesterol / HDL cholesterol	3.66	(1.39)
Hypercholesterolemia (tot chol >6.5 mmol/l, history)	55.0%	
Diabetes (medication, history)	17.5%	
Current/quit smoking	97.0%	
Cholesterol lowering medication	70.7%	
Antithrombotic medication	85.1%	
Antihypertensive medication	52.7%	
Median Agatston score	276	(39-827)

Continuous measures are mean with standard deviation between parentheses. Dichotomous measures are given as percentages. Median Agatston score is given with inter-quartile range in parentheses.

Table 2. Risk factor characteristics per calcium score category

	0	>0-10	>10-100	>100-400	>400
	n=17	n=19	n=37	n=59	n=87
Age (years)*	58.6	59.3	59.9	62.6	64.7
Male sex	59%	58%	81%	76%	80%
BMI (kg/m^2)	26.0	25.0	26.7	26.6	26.4
Systolic blood pressure (SBP) (mmHg)	143.7	147.4	144.8	142.9	150.4
Diastolic blood pressure (DBP) (mmHg)	80.5	85.6	81.6	82.1	82.1
Hypertension (SBP>140, DBP>90, med)	59%	68%	62%	64%	77%
Total cholesterol (mmol/l)	4.55	4.99	4.57	5.04	4.84
Total chol / HDL chol	3.22	3.58	3.75	3.74	3.69
Hypercholesterolemia (tot chol >6.5 mmol/l, history)	35%	53%	49%	58%	61%
Diabetes (medication, history)	19%	11%	17%	14%	21%
Current/quit smoking	100%	100%	100%	94%	96%
Cholesterol lowering med	75%	60%	84%	61%	73%
Antithrombotic med	83%	93%	84%	90%	81%
Antihypertensive med	50%	40%	41%	55%	59%
Median Agatston score	0	5	39	208	985

*only age is significantly different between calcium score groups ($p<0.001$ Kruskal Wallis test); all other characteristics are not significantly different between groups.

Calcium scores

Median Agatston score was 276 (range 0-5135). 36 subjects (16%) had an Agatston score up to 10 and 87 subjects (40%) an Agatston score above 400. In higher calcium score groups age was significantly higher (Table 2). No other significant relations were found.

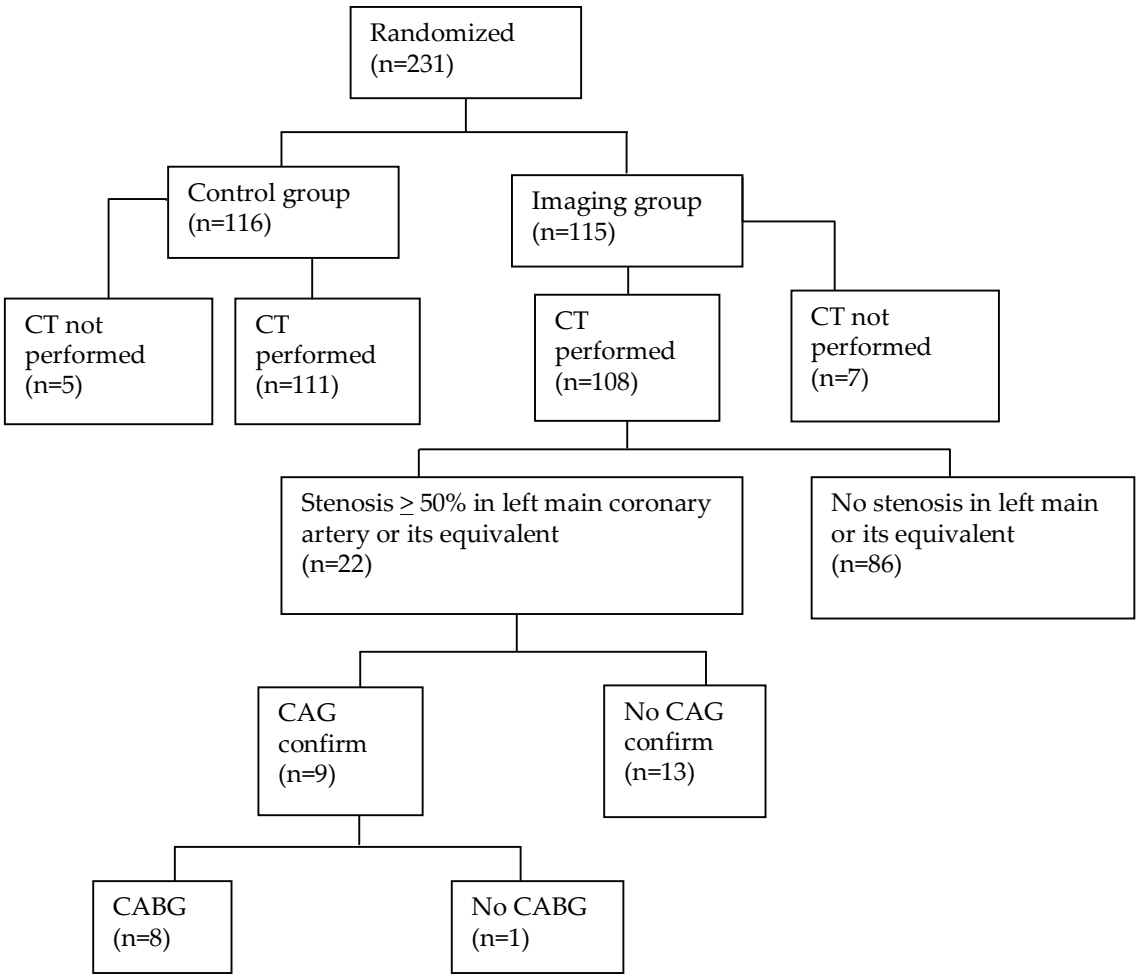

Figure 1: Flow chart of the patients in the GROUND study

Significant left main (equivalent) stenosis

In the screening group 22 subjects showed a significant (equivalent) left main stenosis on cCTA (left main stenosis n=13, left main equivalent stenosis n=9). Sixteen of these 22 patients had an Agatston score above 400, five between 100 and 400. One patient had a very low Agatston score of 8. A significant left main stenosis was confirmed with invasive angiography in 9 subjects; no equivalent stenosis was

confirmed. Eight of these patients underwent uncomplicated CABG surgery. One patient did not undergo CABG surgery since his coronary anatomy was deemed unsuitable. All other patients referred to a cardiologist received either PCI in single significantly stenosed vessels (n=3) or maximal conservative treatment with medication.

Table 3. Distribution of significant left main stenoses, treated with CABG, over calcium score categories

	CABG for significant left main stenosis	
Calcium score category	-	+
0	8	0
>0-10	9	1
>10-100	16	0
>100-400	31	0
>400	36	7
all	100	8

Table 4. Univariable logistic regression: significant left main stenosis eligible for CABG as dependent variable

	OR	95% CI	p-value
Age (years)	1.04	0.94-1.15	0.43
Male sex	$1.7x10^8$	-	1.00
BMI (kg/m^2)	1.03	0.83-1.27	0.82
Systolic blood pressure (SBP) (mmHg)	1.01	0.98-1.04	0.58
Diastolic blood pressure (DBP) (mmHg)	0.95	0.87-1.04	0.27
Hypertension (SBP>140, DBP>90, med)	1.41	0.27-7.39	0.68
Total cholesterol (mmol/l)	1.23	0.71-2.10	0.46
Total chol / HDL cholesterol ratio	1.11	0.66-1.89	0.69
Hypercholesterolemia (tot chol >6.5 mmol/l, history)	0.89	0.21-3.75	0.87
Diabetes (med, history)	6.14	1.38-27.44	0.02
Smoking (current & quit)	0.15	0.01-1.89	0.14
Agatston score (continuous)	1.00	1.00-1.00	0.07
Agatston score >400	12.44	1.47-105.21	0.02

Significant left main (equivalent) stenosis

In the screening group 22 subjects showed a significant (equivalent) left main stenosis on cCTA (left main stenosis n=13, left main equivalent stenosis n=9). Sixteen of these 22 patients had an Agatston score above 400, five between 100 and 400. One

patient had a very low Agatston score of 8. A significant left main stenosis was confirmed with invasive angiography in 9 subjects; no equivalent stenosis was confirmed. Eight of these patients underwent uncomplicated CABG surgery. Table 3 shows the distribution over calcium score categories of these 8 patients. One patient did not undergo CABG surgery since his coronary anatomy was deemed unsuitable. All other patients referred to a cardiologist received either PCI in single significantly stenosed vessels (n=3) or maximal conservative treatment with medication.

Significant associations ($p<0.05$) between cardiovascular risk factors and significant left main stenosis eligible for surgery in the whole intervention group were found only for presence of diabetes using univariable logistic regression (Table 4); presence of diabetes was associated with a 6.1-fold increase (95% CI 1.4-27.4). Agatston score above 400 was also significantly associated with the presence of significant left main stenosis eligible for surgery (OR=12.4: 95% CI 1.5-105.2). Diabetes mellitus and Agatston score above 400 were included in a multivariable logistic regression model. The ROC area of this model was 0.82 (95% CI 0.65-0.98). 40% of the patients with both an Agatston score >400 and diabetes had a significant left main stenosis, eligible for surgery (Table 5).

Table 5. Distribution of occurrence of significant left main stenosis, eligible for CABG, according to both Agatston score (threshold value 400) and diabetic status (in last column mean and standard deviation are given)

		Number of patients	CABG for significant left main stenosis	
Agatston score <400	Diabetes -	57	1	2% (± 13%)
	Diabetes +	8	0	0% (± 0%)
Agatston score >400	Diabetes -	33	3	9% (± 29%)
	Diabetes +	10	4	40% (± 52%)

Smoking was not included in the model despite a P-value below 0.157. The reason was that there was a negative correlation between smoking and the presence of significant coronary artery disease according to the logistic regression analysis, i.e. non-smokers would have a higher risk than smokers. Thus, this was regarded as a chance finding.

Discussion

The results of this study show that in this high-risk group of cardiac asymptomatic peripheral arterial disease patients, coronary calcium scoring may be able to differentiate which patients are more likely to suffer from significant coronary artery disease eligible for surgery. This ability of calcium scoring for the detection of obstructive angiographic coronary artery disease has also been found in cardiac symptomatic patients[19;20]. In a cardiac symptomatic patient group calcium scoring may even be superior to thallium and ECG exercise tests for predicting coronary artery stenosis[21].

The role of coronary calcium scoring as a selection tool of those who need further testing for cardiac disease, such as SPECT, has been mentioned in recent reports on cardiovascular screening in cardiac asymptomatic subjects[22;23]. However, these guidelines are mainly aimed at asymptomatic subjects with cardiovascular risk factors and not yet symptomatic atherosclerosis. The subjects we studied do have symptomatic atherosclerosis and already are at a high risk, which justifies intensive medical therapy. Therefore, calcium scoring is not aimed at determining who needs intensive medical therapy but rather may have a role in selecting patients for further, more extensive tests, such as angiography, and resulting treatment. The relatively cheap and easy calcium scoring scan could possibly function as a gatekeeper for the more expensive and time-consuming further tests. Standard cardiovascular risk factors seem unable to make this subdivision between high-risk patients with a higher or lower risk. In the past thallium scintigraphy instead of calcium scoring has been suggested to be valuable for further selection in patients with PAD[24;25]. Calcium scoring would be another entirely non-invasive test which is faster, less stressful and much less expensive for the patient than thallium testing.

In case a screening endeavor is undertaken a treatable disease has to be detected. Early treatment of this disease also needs to improve patient prognosis. Studies in cardiac symptomatic patient groups studied the relation between calcium score and obstructive disease [19;20;26]. We limited our definition of significant obstructive disease to left main (equivalent) stenosis since only significant left main (equivalent) stenosis is a class I indication for treatment with CABG, even the case in absence of symptoms, in the standards set by the American Heart Association [14]. Although CABG is not without a certain morbidity and mortality risk, early

treatment of a significant left main stenosis can probably prevent sudden cardiac death. However, it is unknown if a screening endeavor like undertaken in this study is changing life expectancy and if it is cost-effective since follow-up data is lacking. Especially in light of the COURAGE study, in which stable coronary heart disease patients were equally well off being treated by PCI or by optimal medical treatment, data on these issues are needed[27]. Calcium scoring as an initial test was only cost-effective in symptomatic patient groups with a significant disease prevalence below 70%.[28] Therefore, careful selection of the screening population is necessary. We chose to study cardiac asymptomatic PAD patients. In cardiac asymptomatic PAD patients the mortality risk is smaller than in PAD patients with symptoms of cardiac disease but the risk is still increased compared to subjects without PAD[5;7]. Our results suggest that CT calcium scoring may be used as a pretest to select those patients that need to undergo morphologic tests, such as cCTA and invasive angiography.

A small percentage of the patients in our study had no to very little calcifications in their coronary arteries despite their high-risk status. Several earlier calcium scoring studies in cardiac symptomatic patients show that low calcium scores exclude the occurrence of significant findings. Janssen et al. reported that a calcium score below 11 precludes wall motion abnormalities during dobutamine stress MR and Moser et al. showed that with calcium scores below 100 SPECT is consistently negative for ischemic changes[29;30]. Other studies showed that exclusion of any calcium is highly accurate in ruling out obstructive disease[31;32]. However, in our study we did detect a significant left main stenosis due to soft plaques in one patient with an Agatston score below 10. Rubinshtein et al. even found significant coronary stenoses in 12% of patients with chest pain syndrome and a calcium score below 100 [33]. Therefore, even with a low calcium score significant disease with a treatment indication is not completely ruled out.

This study has several limitations. The sample size was relatively small. Thereby, only in the subgroup that also underwent cCTA we had information on the presence of a significant left main (equivalent) stenosis. The significant correlations we found had large confidence intervals and could have been chance findings. Thereby, the absence of significant differences in risk factors could also have been due to the small sample size.

Furthermore, we did not have any follow-up information yet. Therefore, the implications of our findings are unknown. It is uncertain if imaging findings are related to the eventual occurrence of events, such as death, and if early treatment of significant findings improves prognostics. Long-term follow-up results of the ongoing GROUND study hopefully will provide answers regarding the occurrence of events.

Despite the small sample size and the lack of follow-up, this study is one of the first efforts undertaken to screen high-risk patients for cardiac disease with both CT calcium scoring and cCTA and this study does show promising results for risk stratification in a high-risk patient group. In conclusion, calcium scores and diabetic status may help in predicting the occurrence of significant coronary artery disease, eligible for surgery, in cardiac asymptomatic PAD patients.

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Chapter 6

Measurement of epicardial and peri-coronary adipose tissue with cardiac computed tomography in patients suspected of coronary artery disease

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Abstract

Objective: Adipose tissue surrounding coronary arteries may contribute to the development of coronary atherosclerosis given its localisation and potential for local production of inflammatory cytokines. We compared various measurements for quantifying epicardial adipose tissue (EAT) and peri-coronary fat using cardiac CT. Additionally, we estimated their relationship with obesity and metabolic syndrome in patients suspected of coronary artery disease (CAD).

Methods: EAT and peri-coronary fat measurements were performed on cardiac multi-slice CT scans in 60 patients (aged 50-70 yrs) referred for coronary angiography. EAT was measured as thickness on the right ventricular free wall, as area at the base of the ventricles, and as volume. Peri-coronary fat was assessed as thickness and cross-sectional area surrounding the three main coronary arteries. Linear regression analysis was used to assess the relation of EAT and peri-coronary fat with obesity and metabolic syndrome (ATP III criteria).

Results: Volumetric EAT measurements showed good reproducibility with low coefficients of variation (CVs) varying between 3.0% and 5.0%. Measurements of EAT and peri-coronary fat thickness and area were moderately reproducible (CVs 11.0%-23.4%). The amount of EAT and peri-coronary fat (per standard deviation) was related with obesity (BMI ≥30 kg/m²) (β 1.24; 95% CI 0.66; 1.81) and metabolic syndrome (β 0.81; 95% CI 0.28; 1.33).

Conclusions: Volumetric quantification of EAT using cardiac CT is highly reproducible compared to more simple measurements as EAT and peri-coronary fat thickness and area. The quantity of EAT and peri-coronary fat is related with the presence of obesity and metabolic syndrome in patients suspected of CAD.

Introduction

Abdominal obesity is associated with an increased risk of coronary artery disease (CAD)[1]. Dyslipidemia, hypertension and insulin resistance are important risk factors associated with obesity[2]. The clustering of these risk factors is often referred to as metabolic syndrome[3]. Moreover, abdominal adipose tissue is able to produce large quantities of tumor necrosis factor-alpha (TNF-α), interleukine-6 (IL-6), free fatty acids (FFA), plasminogen activator inhibitor-1 (PAI-1) and adipocytokines such as adiponectin[4-6]. Increased plasma concentrations of TNF-α, IL-6, FFA, and PAI-1 and decreased concentrations of adiponectin lead to accelerated atherosclerosis, plaque instability, and arterial thrombosis[6-8].

Adipose tissue surrounding the coronary arteries, often called epicardial adipose tissue (EAT), may also act as an endocrine organ given the production of a comparable pattern of adipocytokines[9;10]. It could be hypothesized that adipocytokines produced by fat surrounding the coronary arteries might contribute to the amplification of vascular inflammation and pro-atherogenic processes from 'outside-to-inside' the vessel wall (Figure 1)[11;12].

To further clarify the possible relation between fat surrounding coronary arteries and the development of CAD, it is important to reliably quantify this adipose tissue. In a few studies adipose tissue around the heart has been measured by magnetic resonance imaging, computed tomography (CT) or echocardiography[13-18]. Furthermore, there are differences in anatomic description (pericardial versus epicardial fat) and measurement techniques (volume or thickness)[13;16;18]. A measurement of adipose tissue directly surrounding the coronary arteries has not been published. Conceptually, this peri-coronary fat might be most interesting because of its close anatomic relation with the coronary arteries.

CT provides a more accurate quantification of adipose tissue due to its higher spatial resolution compared to magnetic resonance imaging and ultrasound. However, limited methods for measuring fat surrounding coronary arteries using cardiac CT have been explored[13].

In this cross-sectional study, we compared the reproducibility of various methods for quantifying EAT and peri-coronary fat using cardiac CT in patients suspected of CAD referred for angiography. In addition, we estimated the

relationship of the quantity of EAT and peri-coronary fat with obesity and metabolic syndrome and its components.

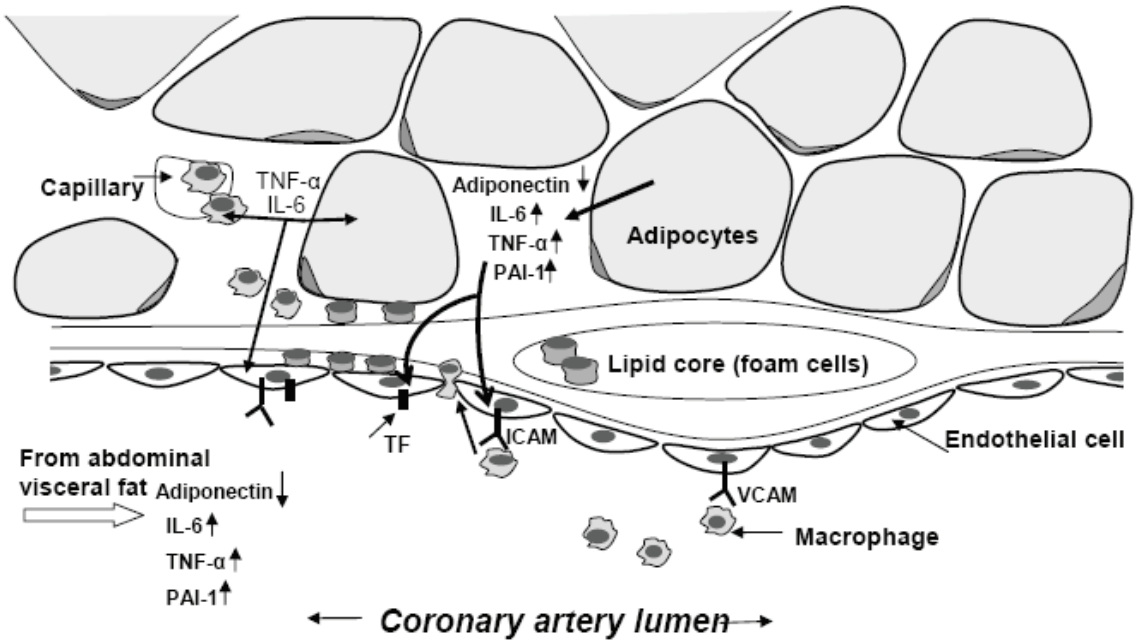

Figure 1. Potential role of adipocytes in the process of coronary atherosclerosis, plaque instability, and arterial thrombosis. TNF-α, tumor necrosis factor-alpha; IL-6, interleukine-6; PAI-1, plasminogen activator inhibitor-1; TF, tissue factor; ICAM, VCAM, endothelial adhesion molecules.

Methods

Study settings, participants and design

Patients originated from a diagnostic study at the University Medical Centre Utrecht designed to establish the diagnostic accuracy of multi-slice computed tomography coronary angiography (CTA) compared to conventional coronary angiography (CAG) in the detection of significant coronary obstruction. Patients (n = 60) were referred for diagnostic coronary angiography or percutaneous coronary intervention because of stable angina pectoris (n = 48) or unstable angina pectoris (n = 12)[19;20]. The inclusion criteria were: aged 50-70 years, and stable sinus rhythm. Patients with previous PTCA or CABG, serum creatinine levels >140 μmol/l, or known iodine-based contrast allergy were not included. CTA was performed in all patients within a month before or after CAG. The Medical Ethics Committee approved the study and all participants gave their written informed consent.

Clinical information was obtained using a standardised health questionnaire. Height, body weight, and blood pressure were measured. Fasting blood was sampled to determine lipid, glucose and creatinine levels. Diabetes mellitus was defined as self-reported diabetes mellitus. Metabolic syndrome was diagnosed according to the Adult Treatment Panel III (ATP III) criteria as the presence of three or more metabolic abnormalities[3]. Because waist circumference was not available, a BMI of ≥30 kg/m² was used as determinant for obesity[21]. By visual assessment of CAGs by cardiologists the severity of CAD was classified as single, two or three vessel disease. A ≥50% luminal diameter stenosis was regarded as significant[22].

CT technique and image analyses

EAT and peri-coronary fat were quantified on ECG-gated diagnostic cardiac CT scans. CT studies were performed on a 64 detector-row CT scanner (Brilliance 64, Philips Medical Systems, Cleveland, OH, USA). Scan duration time was 7-10 s. Average heart rate during image acquisition was 56 ± 10 beats/min. Standard coronary imaging protocols were applied including the use of intra-venous beta-blockers for patients with heart rates >65 beats/min (unless contraindicated) and image acquisitions were performed during a breath-hold in inspiration. Imaging parameters were slice collimation of 64 mm x 0.625 mm, gantry rotation time of 420 ms, tube voltage of 120 kV, and tube current of 900 mAs. Contrast agent used was iopromide (Schering AG, Berlin, Germany), which was injected intravenously (1.6-2.0 g iodine/s depending on the patient's body weight).

Measurements were performed in the most motionless phase of the cardiac cycle, which was most frequently a mid-diastolic phase, with retrospective cardiac gating at 70-80% of the R-R interval. The window settings were adjusted to properly visualise the adipose tissue and the pericardium.

To establish the intra- and interobserver variability for measurement techniques of EAT and peri-coronary fat, all cardiac CT scans were analysed independently by two observers who were blinded for their own and each others results and for patient characteristics.

EAT quantification

EAT was defined as the adipose tissue between the surface of the heart and the visceral layer of the pericardium (visceral epicardium).

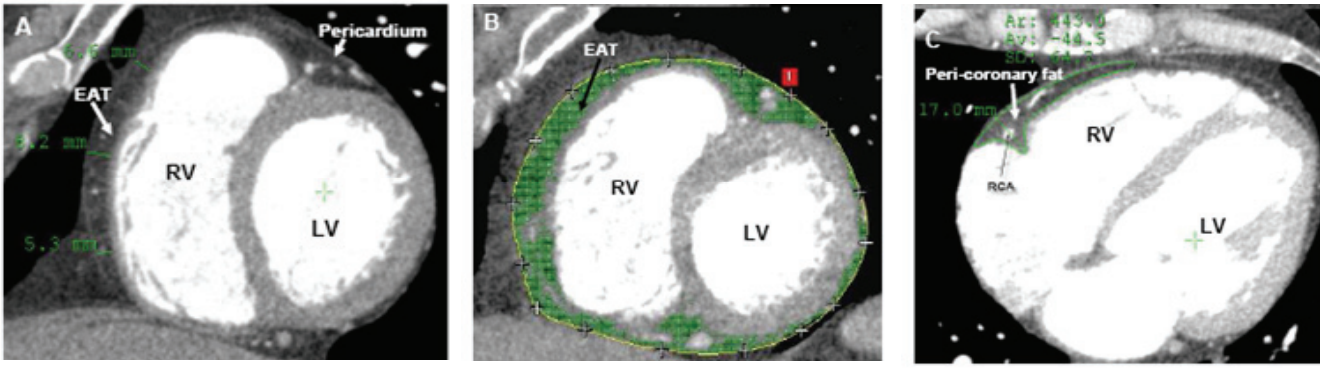

Figure 2. Epicardial adipose tissue (EAT) and peri-coronary fat measurements. (A) EAT RV thickness (green) (inferior, centre, and superior) at basal level of the ventricles. (B) Region of interest (ROI) (yellow) on a cross-sectional image to determine the EAT area (green). EAT volume is the sum of the EAT areas on cross-sectional images. (C) Peri-coronary fat thickness and area (green).

EAT thickness

EAT thickness (mm) was determined on the right ventricular (RV) anterior free wall. Measurements were performed at the base of the ventricles (basal level) on short-axis views of a regular Philips CT workstation (Figure 2A). Basal level was defined as the level at the base of the ventricles.16 Three measurements of EAT thickness were made: inferior, centre and superior, chosen at 25%, 50% and 75% level of the RV wall, respectively, from the visceral epicardium to the outside of the myocardium and perpendicular to the surface of the heart. The mean of the three measurements (referred to as 'EAT RV thickness') was used for analyses.

EAT area

EAT area (cm2) was measured using two dedicated software programs (Extended Brilliance Workspace and Easy Vision, Philips Medical Systems, Best, The Netherlands). Measurements were performed on short-axis views of 2-mm-slice-thickness and 3-mm-intersection gaps. The stack of the short-axis views started at the apex just below the fibrous pericardium and extended until the centre of the left atrium. One section of the short-axis image, at the base of the ventricles (basal level),

was used to determine 'EAT area at basal level'. An EAT area measurement was assessed by tracing a single region of interest (ROI) containing heart and EAT (Figure 2B). ROI's were placed at the visceral epicardium to exclude pericardial fluid. A density range between -200 and -30 Hounsfield Units was used to isolate adipose tissue.

EAT volume

EAT volume (cm3) was obtained as the sum of the EAT areas on short-axis images using the same software programs, short-axis views and range of Hounsfield Units as for 'EAT area at basal level'. A cursor pointer was used to trace a ROI (Figure 2B). The computer software program produced an accurate volume of EAT by adding up the EAT areas and taking into account slice thickness and intersection gap.

Firstly, 'EAT truncated volume' was obtained as the sum of the EAT areas made of 20-25 sections extending from below the apex up to a cut-off point 1 cm (two sections) above the last visible segment of the left coronary artery (LCA). Second, 'EAT total volume' was assessed by adding up EAT areas of 4-6 sections (until the centre of the left atrium) to the 'EAT truncated volume'.

Peri-coronary fat quantification

Peri-coronary fat was defined as the adipose tissue between the surface of the heart and the visceral epicardium directly surrounding the main coronary arteries.

Peri-coronary fat thickness

Peri-coronary fat thickness (mm) was quantified on axial views of a regular Philips CT workstation. In order not to overestimate the peri-coronary fat due to obliquity, thickness measurements were performed on images in which the axial sections were perpendicular to the surface of the heart. In each of the regions of the right coronary artery (RCA), LCA and left circumflex (LCX), maximal fat thickness assessed as the largest distance from myocardium to visceral epicardium was determined (Figure 2C). Mean thickness of the peri-coronary fat surrounding the three coronary arteries was used for analyses ('peri-coronary fat thickness').

Peri-coronary fat area

Separately for the three coronary arteries, the cross-sectional area (cm2) of fat surrounding the coronary artery was determined on axial sections by tracing a ROI (Figure 2C). In case fat area covered both the RCA and the LCA region, ROI extended halfway between both regions, and the area was measured for the involved coronary artery. Mean cross-sectional area surrounding the three main coronary arteries was used for analyses ('peri-coronary fat area').

Relationship of EAT and peri-coronary fat with obesity and metabolic syndrome

To evaluate the relation of EAT and peri-coronary fat quantity with obesity and metabolic syndrome and its components, we used (a) the most reproducible measurement (EAT truncated volume); (b) the measurement most commonly used in previous studies (EAT RV thickness); and (c) the most interesting measurement from a pathophysiological point of view (peri-coronary fat thickness).

Statistical analysis

Mean differences with 95% confidence intervals (CI) of EAT and peri-coronary fat measurements between two observers or between repeated measurements of one observer were tested with the paired t-test (two-sided). To assess the intra- and interobserver reproducibility, a coefficient of variation (CV) was calculated using the formula: (standard deviation (S.D.) of the differences between the measurements/overall mean of the measurements) x 100. Repeatability coefficient (RC) was computed as 2 x (S.D. of the differences between two measurements). The limits of agreement were calculated as mean difference ± (1.96 x (S.D. of the differences)).

The relationship of the amount of EAT and peri-coronary fat with obesity and metabolic syndrome and its components was determined with linear regression analyses. To compare the various dimensions of fat measurements, we determined the age- and gender-adjusted regression coefficient beta (β) (95% CI) corresponding to a 1 S.D. change in fat measurement. Variables were log-transformed if they were skewly distributed. To adjust mean EAT and peri-coronary fat levels for age and gender differences between the number of components of metabolic syndrome we used analysis of covariance (ANCOVA, general linear model). To reduce bias and

increase statistical efficiency, missing values (HDL-cholesterol (n = 8), triglycerides (n = 8), fasting glucose (n = 2), systolic (n = 6) and diastolic (n = 6) blood pressure) in the data were completed by regression imputation[23;24].

Results

Baseline characteristics

Patients (n = 60) had a mean age of 61 ± 5 years (range 50-70 years) and the mean body mass index (BMI) was 27 ± 4 kg/m2 (Table 1). Mean creatinine clearance (Cockroft-Gault) was 87 ± 23 ml/min/1.73m2. Dyslipidemia (LDL-cholesterol >2.5 mmol/l or use of lipid-lowering agents) was present in 57 patients (93%).

Table 1. Baseline characteristics of the study population (n = 60)

Characteristics	Data
Age, years[1]	61 ± 5
Male gender, n (%)	44 (73)
Body mass index (kg/m^2)[1]	27 ± 4
Systolic blood pressure (mm Hg)[1]	152 ± 23
Diastolic blood pressure (mm Hg)[1]	82 ± 13
Creatinine clearance (ml/min/1.73m^2)*	
≥ 90 ml/min/1.73m^2, n (%)	23 (38)
60-89 ml/min/1.73m^2, n (%)	33 (55)
30-59 ml/min/1.73m^2, n (%)	4 (7)
Current smoking†, n (%)	21 (35)
Diabetes mellitus‡, n (%)	7 (12)
Metabolic syndrome ATP III, n (%)	22 (37)
Laboratory measurements	
Fasting glucose (mmol/l)[2]	5.9 (5.0–6.4)
Total cholesterol (mmol/l)[2]	4.4 (3.8–5.1)
LDL-cholesterol (mmol/l) §, [2]	2.25 (1.87–3.11)
HDL-cholesterol, mmol/l[2]	1.26 (1.08–1.58)
Triglycerides, mmol/l[2]	1.38 (1.07–2.19)
Medications	
Blood pressure-lowering agents, n (%)	53 (88)
Lipid-lowering agents, n (%)	45 (75)
Parameters of coronary artery disease	
Stable angina pectoris, n (%)	48 (80)
History of myocardial infarction, n (%)	13 (22)
Severity of coronary artery disease‖	
No significant affected vessels, n (%)	11 (18)
1-Vessel disease, n (%)	28 (47)
2-Vessel disease, n (%)	11 (18)
3-Vessel disease, n (%)	10 (17)

[1] Mean ± S.D.
[2] Median with interquartile range
* Categorisation of creatinine clearance (Cockroft-Gault) according to K/DOQI criteria for the degree of renal insufficiency
† Still smoking or recently (<1 month before study inclusion) stopped smoking
‡ Self-reported diabetes mellitus
§ Calculated by use of Friedewald`s formula
‖ According to conventional coronary angiograms, ≥50% is defined as significant stenosis

EAT and peri-coronary fat quantification and reproducibility

The overall mean ± S.D. for intra- and interobserver measurements of EAT and peri-coronary fat are depicted in Table 2. Peri-coronary fat thickness was 15.9 ± 3.0 mm for the RCA region, 5.7 ± 2.1 mm for the LCA region, and 11.0 ± 2.1 mm for the LCX region.

EAT truncated volume and EAT total volume were highly reproducible (interobserver CVs 4.8% and 5.0%, respectively). EAT area at basal level and EAT RV thickness appeared to be moderately reproducible; CVs varied between 11.0 and 16.9%. Interobserver repeatability coefficient was 1.6 mm for EAT RV thickness with regard to an overall mean of 4.8 mm. Peri-coronary fat thickness and area measurements were moderately (CV 13.1%) and poorly reproducible (CV 23.4%), respectively (Table 2). Bland-Altman analysis[25] showed similar results (data not shown). For all measurements, Pearson's correlation coefficients revealed no relation between intra- and interobserver differences and the range of fat quantity (data not shown).

Relationship of EAT and peri-coronary fat with obesity and metabolic syndrome

An age- and gender-adjusted association was found for the quantitative estimates of EAT and peri-coronary fat (per S.D.) with obesity (BMI ≥30 kg/m²) (for EAT RV thickness: β 0.88, 95% CI (0.27; 1.49), for EAT truncated volume: 1.18, (0.61; 1.75) and for peri-coronary fat thickness: 1.24, (0.66; 1.81) (Table 3). A comparable age- and gender-adjusted relation with metabolic syndrome was found for EAT truncated volume (β 0.70, 95% CI (0.18; 1.23) and peri-coronary fat thickness (0.81, (0.28; 1.33)). EAT RV thickness was less and non-significantly associated with metabolic syndrome (0.30, (-0.24; 0.85)) (Table 3). Fat surrounding the coronary arteries (mean ± standard error) gradually increased with the number of components (0 or 1—4 or 5): EAT truncated volume (90.5 ± 13.1 to 144.6 ± 15.6 cm3, p-value for trend 0.038), and peri-coronary fat thickness (9.7 ± 0.5 to 12.1 ± 0.6 mm, p-value for trend 0.015).

Table 2. Distribution and intra- and interobserver reproducibility of epicardial adipose tissue (EAT) and peri-coronary fat measurements (n = 60)

	Intra-observer					Inter-observer				
	Mean ± SD*	Mean difference (95% CI)	(LLA; ULA) †	CV‡	RC§	Mean ± SD*	Mean difference (95% CI)	(LLA; ULA) †	CV‡	RC§
EAT										
EAT RV thickness, mm	4.9 ± 2.0	0.05 (-0.10;0.19)	(-1.06;1.15)	11.5%	1.1	4.8 ± 1.9	0.20 (-0.01;0.41)	(-1.39;1.79)	16.9%	1.6
EAT area at basal level, cm^2	35.2 ± 16.5	1.28 (0.27;2.28)	(-6.32;8.87)	11.0%	7.8	35.5 ± 16.6	0.61 (-0.73;1.95)	(-9.55;10.77)	14.6%	10.4
EAT truncated volume, cm^3	110.2 ± 46.7	0.88 (0.02;1.73)	(-5.60;7.35)	3.0%	6.6	110.1 ± 46.6	1.15 (-0.21;2.51)	(-9.16;11.47)	4.8%	10.5
EAT total volume, cm^3	118.2 ± 50.3	-0.13 (-1.07;0.82)	(-7.32;7.06)	3.1%	7.3	117.8 ± 50.2	0.68 (-0.84;2.21)	(-10.89;12.26)	5.0%	11.8
Peri-coronary fat										
Peri-coronary fat thickness, mm	10.9 ± 1.9	0.54 (0.20;0.88)	(-2.03;3.11)	12.0%	2.6	10.9 ± 1.9	0.64 (0.27;1.00)	(-2.15;3.43)	13.1%	2.8
Peri-coronary fat area, cm^2	2.3 ± 1.1	0.12 (0.03;0.21)	(-0.56;0.81)	15.2%	0.7	2.1 ± 1.0	0.41 (0.28;0.53)	(-0.56;1.38)	23.4%	1.0

* Overall mean of the measurement ± standard deviation, S.D.

† LLA, lower limit of agreement; ULA, upper limit of agreement

‡ CV, coefficient of variation, calculated as S.D. of the differences between the measurements/overall mean, multiplied by 100

§ RC, coefficient of repeatability, calculated as 2 x S.D. of the differences between the measurements

Table 3. Measurements of epicardial adipose tissue (EAT) and peri-coronary fat in relation to obesity and metabolic syndrome and its components (n = 60)

	EAT truncated volume (1 SD = 46.17 cm^3)	EAT RV thickness (1 SD = 1.88 mm)	Peri-coronary fat thickness (1 SD = 1.86 mm)
	β (95% CI) *	β (95% CI) *	β (95% CI) *
Continuous variables			
BMI per 1.0 kg/m^2	0.17 (0.11; 0.22)	0.14 (0.08; 0.20)	0.19 (0.13; 0.24)
Systolic blood pressure per 10 mm Hg	-0.05 (-0.17; 0.06)	-0.02 (-0.14; 0.09)	0.02 (-0.09; 0.14)
Fasting glucose per 1.0 mmol/l†	0.33 (-0.50; 1.16)	-0.05 (-0.88; 0.78)	0.38 (-0.46; 1.22)
Triglycerides per 0.5 mmol/l†	0.16 (-0.34; 0.67)	-0.13 (-0.63; 0.38)	0.25 (-0.26; 0.76)
HDL-cholesterol per 0.1 mmol/l	-0.01 (-0.09; 0.07)	-0.05 (-0.13; 0.03)	-0.06 (-0.13; 0.02)
Dichotomous variables			
Diabetes mellitus‡	0.36 (-0.44; 1.16)	0.77 (-0.01; 1.55)	0.39 (-0.42; 1.20)
Metabolic syndrome ATPIII	0.70 (0.18; 1.23)	0.30 (-0.24; 0.85)	0.81 (0.28; 1.33)
BMI ≥30 kg/m^2	1.18 (0.61; 1.75)	0.88 (0.27; 1.49)	1.24 (0.66; 1.81)
≥130 mm Hg systolic or ≥85 mm Hg diastolic and/or BP lowering agents	0.50 (-0.54; 1.54)	0.30 (-0.74; 1.35)	0.34 (-0.72; 1.40)
fasting glucose ≥5.6 mmol/l or diabetes mellitus[c]	0.48 (-0.03; 0.99)	0.19 (-0.33; 0.71)	0.31 (-0.21; 0.83)
triglycerides ≥1.70 mmol/l	-0.01 (-0.57; 0.56)	-0.29 (-0.84; 0.27)	0.19 (-0.38; 0.76)
HDL-cholesterol <1.03 mmol/l (men) or <1.29 mmol/l (women)	-0.08 (-0.70; 0.53)	0.10 (-0.52; 0.71)	0.18 (-0.45; 0.80)

β: Beta regression coefficient. For continuous variables, β indicates the absolute change in S.D. of the corresponding fat measurement associated with an increase of one unit in the metabolic syndrome component (e.g. increase of 1 kg/m^2 in BMI leads to a change in EAT truncated volume of 0.17 S.D., meaning 0.17 x 46.17 = 7.85 cm^3). For dichotomous variables, β should be interpreted as follows: β x S.D. is the difference between groups (e.g. patients with metabolic syndrome have a 0.70 x 46.17 = 32.32 cm^3 higher EAT truncated volume than patients without metabolic syndrome).

* Adjusted for age, and gender

† β (95% CI) derived using log-transformation

‡ Self-reported diabetes mellitus

Discussion

Quantifying EAT volume with cardiac CT scanning is highly reproducible compared to more simple measurement techniques, such as EAT and peri-coronary fat thickness and area. The amount of this adipose tissue was associated with obesity and metabolic syndrome in patients clinically suspected of CAD.

Adipose tissue surrounding the coronary arteries (EAT or peri-coronary fat) has the potential for local production of inflammatory cytokines[9;10;26]. An increased quantity

or dysfunction of this adipose tissue may contribute to a pro-atherogenic environment on the outside of coronary arteries affecting vascular function. Thus far, the direct contribution of fat surrounding coronary arteries to the development of CAD is still under investigation[14;27]. For investigating this relationship it is important to establish a reliable quantification of this adipose tissue.

CT technique allows for the precise measurement of fat surrounding coronary arteries, because the fibrous pericardium can be easily visualised due to the high spatial resolution. So far, limited methods for measuring this adipose tissue using cardiac CT have been published[13]. Previous studies evaluated the use of cardiac CT for the assessment of pericardial fat volume instead of EAT volume and used the centre of the right pulmonary artery[18] and the atrial appendage[17] as cut-off points for the extent of volume. Pericardial fat includes EAT and paracardial fat separated by the fibrous pericardium. Conceptually, measuring EAT or peri-coronary fat (surrounding the coronary arteries) might be more important than quantifying pericardial fat[17;18]. In the present study, we have used various dimensions (thickness, area and volume) to quantify EAT and peri-coronary fat with cardiac CT in a relevant population.

Our results demonstrate that volumetric quantification of EAT using cardiac CT is feasible and yields superior reproducibility compared to thickness and area measurements. This is consistent with a recent study showing that a magnetic resonance imaging-based volumetric approach was highly reproducible (CV 6%)[15]. In our study, the most reproducible measurement was the EAT truncated volume. Because the cut-off point was set at 1 cm above the last segment of the LCA, all fat volume surrounding the coronary arteries was evaluated. It should be noted that volumetric assessment is time consuming, requires an advanced cardiac imaging workstation and should be done by a skilled observer with sufficient knowledge of cardiac anatomy. Recently, EAT thickness measurement on the RV wall has been performed on short-axis views of CT-scans to evaluate EAT distribution instead of reliability[13]. In the present study, we established a moderate reproducibility for EAT RV thickness measured with CT. This is probably due to the little variation in recognising basal level and tracing the cursor pointer to measure thickness in millimetres. However, this method is more easy to perform and less time consuming compared to the labour-intensive determination of EAT volume with cardiac CT.

Previous studies mainly measured EAT thickness using echocardiography[14;16]. The ability to measure EAT with echocardiography is modest compared to the use of CT; it can not give an adequate window of all cardiac segments and moreover is highly dependent on acoustic windows, which are often inadequate for subtle assessments in obese patients[13]. Remarkably, a low interobserver CV (3%) for echocardiographic EAT RV thickness was reported[16]. EAT is accurately visualised on CTA, but in contrast to echocardiography, CTA requires the use of an intravenous contrast agent and a radiation dose of 5-15 mSv.

In line with previous findings[28], the amount of EAT and peri-coronary fat was strongly associated with obesity in patients suspected of CAD. Adipose tissue is a major driver of insulin resistance, an essential pathophysiological feature for the development of metabolic disorders, including e.g. hyperglycaemia, hypertension and low HDL-cholesterol[2;29]. Presence of metabolic syndrome[3] could therefore be seen as an estimation of the insulin resistance state. In an obesity-related insulin resistant state, adipocytes produce large quantities of pro-atherogenic factors (e.g. TNF-α, IL-6, PAI-1)[4-6]. In agreement with previous studies showing that echocardiographic EAT thickness was related with metabolic abnormalities[28], and insulin resistance[30] in obese subjects, our results indicate that EAT and peri-coronary fat accumulation cluster with metabolic syndrome and its components in patients suspected of CAD. This may implicate that adipose tissue directly surrounding coronary arteries may be involved in pathophysiological processes leading to the development of coronary atherosclerosis seen in obesity, insulin resistance and metabolic syndrome.

Some limitations of this study have to be taken into account. This is a cross-sectional study, which means that only assumptions about possible etiological relationships can be made. Moreover, due to the sample size, the confidence intervals are quite wide indicating that the point estimates for the relationship of EAT and peri-coronary fat with obesity and metabolic syndrome and its components are less precise. Furthermore, waist circumference was not measured, thus we could not evaluate the relationship between EAT/peri-coronary fat and abdominal obesity. However, when waist circumference is missing, a BMI ≥30 kg/m^2 is recommended as determinant for obesity to assess the presence of metabolic syndrome[22], and both are indicators of a high cardiovascular risk in patients with cardiovascular disease[31].

Finally, data were obtained in routine medical care. Markers of dysfunction of fat surrounding coronary arteries (IL-6, TNF-α, PAI-1) were not measured in fat samples, and leptin and fasting insulin were not measured. Future studies are needed to reveal the etiologic relation between the quantity and the quality of EAT or peri-coronary fat and CAD.

In conclusion, volumetric quantifications of EAT using cardiac CT were highly reproducible compared to more simple thickness and area measurements of EAT and peri-coronary fat. The quantity of adipose tissue surrounding coronary arteries is related with obesity and metabolic syndrome in patients clinically suspected of CAD.

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Chapter 7

Peri-coronary Epicardial Adipose Tissue is related to Cardiovascular Risk Factors and Coronary Artery Calcification in Post Menopausal Women

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Abstract

Aim: To determine whether peri-coronary Epicardial Adipose Tissue (EAT) is associated to vascular risk factors and coronary atherosclerosis.

Methods and results: In this study 573 healthy postmenopausal women underwent a cardiac CT scan to assess coronary calcification. Peri-coronary EAT thickness was measured in the areas of right (RCA), left anterior descending (LAD) and circumflex coronary artery (LCX). Average EAT thickness was 16.5±4.3 mm (range 5.9, 34.6) in the RCA area, 6.4±2.2 mm (range 2.0, 14.0) in the LAD area and 10.8±3.0 mm (range 2.8, 29.1) in the LCX area. Overall average thickness was 11.2±2.2 mm (range 5.4, 19.1). EAT was positively related to age ($p=0.002$). In age-adjusted linear regression models EAT was positively related to weight ($p<0.001$), waist circumference ($p<0.001$), waist to hip ratio ($p<0.001$), body mass index ($p<0.001$), glucose ($p<0.001$), triglycerides ($p=0.001$), use of anti hypertensive drugs ($p=0.007$), systolic blood pressure ($p=0.034$), and inversely to HDL cholesterol ($p=0.005$). In multivariable models age, weight, waist circumference, smoking and glucose were the main determinants of EAT. EAT showed a graded relation with coronary calcification ($p=0.026$).

Conclusion: EAT is strongly related to vascular risk factors and coronary calcification. Our findings support the hypothesis that EAT affects coronary atherosclerosis and possibly coronary risk.

Introduction

Visceral adipose tissue is an important indicator of cardiovascular risk.[1-5] Abdominal obesity has been shown to be a stronger predictor of cardiovascular risk than increased body mass index.[6] This was found to be true for men and women, in the young and the old and across populations of different ethnic origin.[7]

Epicardial adipose tissue (EAT) is a layer of visceral fat between the myocardium and the pericardium (Figure 1a) and takes up approximately 22% of total heart weight.[8] EAT, as well as intra-abdominal fat, appears to originate from the same brown adipose tissue of infancy and is a rich source of bioactive molecules directly surrounding the coronary arteries.[9]

Coronary arterial calcification (CAC) is a known marker of coronary atherosclerosis[10], which is considered an excessive inflammatory and proliferative process inside the vascular wall.[11;12] There is growing evidence that the presence of inflammatory mediators in the tissues surrounding the epicardial coronary arteries plays an important role in this process.[13-16] It is therefore conceivable that EAT contributes to the local development of atherosclerosis and the occurrence of cardiovascular events.

The few studies to date that assessed EAT predominantly used echocardiography, demonstrating that right ventricular EAT is significantly related to waist circumference, diastolic blood pressure, left ventricular mass and high levels of insulin.[1;17;18] Chaowalit et al. were not able to confirm these findings.[19] It has also been reported that there is a significant association of average maximal EAT thickness on the right ventricle and whole-body glucose uptake, even when adjusted for body mass index (BMI) and waist circumference.[20] In addition, the amount of all the (peri- and epicardial) adipose tissue surrounding the heart has been related to the severity of coronary artery disease, assessed by coronary angiography.[21] The epicardial adipose tissue and, in particular, the adipose tissue directly surrounding the coronary arteries rather than all the adipose tissue surrounding the heart might affect the development of atherosclerosis. However, the relation between only the epicardial adipose tissue that directly surrounds the coronary arteries and coronary atherosclerosis has not yet been addressed.

The current study addressed whether peri-coronary EAT is associated with cardiovascular risk factors and coronary artery calcification.

Methods

Study population

We used data from a cross-sectional study among 573 healthy postmenopausal women. These women were selected from participants PROSPECT study, 1 of the 2 Dutch cohorts participating in the European Prospective Investigation into Cancer and Nutrition.[22] In PROSPECT, 17 357 healthy participants of a nationwide population-based breast cancer screening program, aged 49 to 70 years, living in Utrecht and surroundings, were enrolled between 1993 and 1997. Between October 2002 and April 2004, a re-examination was planned in a sample to investigate the prognostic value of age at menopause on cardiovascular disease (CVD) risk. For this purpose, 6612 women of the total of 17 357 were excluded because of death, further participation in PROSPECT or in other studies, absence of written informed consent, or emigration. Other reasons for exclusion were premenopausal state (n=1309), missing data on menopausal status (n=2105), or use of oral contraceptives or postmenopausal hormone therapy in the year before or after the last menstruation (n=1487), as this precludes accurate estimation of age at menopause. Of 5844 eligible women, a random selection of 1996 women was invited by a personal letter from the principal investigator of PROSPECT, and 1000 (50.1%) answered positively. Of these 1000 women, 573 women were randomly selected for CAC measurement. During the conduct of the study, the possibility of performing CAC measurements came about to study menopausal aspects in relation to coronary atherosclerosis. From a logistical aspect CAC measurement appeared to be possible in 573 women. There was no a priori sample size estimation for EAT, since the possibility for doing EAT measurements came after the scans had been made.

The study complies with the Declaration of Helsinki, the Medical Ethical Committee of the University Medical Centre Utrecht approved the study and written informed consent was obtained from all participants.

Current cardiovascular drug use (blood pressure lowering, lipid lowering and glucose lowering drugs) was assessed by asking women to bring all packages to the study centre. Smoking behaviour, medical history and cardiovascular family history were assessed by a questionnaire. Height and weight were measured and body mass index was calculated as weight divided by height squared (kg/m2). Waist-to-hip ratio was assessed. Systolic and diastolic blood pressures were measured at both arms

with an automated and calibrated blood pressure device (DINAMAP™ XL, Critikon, Johnson & Johnson, Tampa, Florida, USA) with the subject in supine position. A venous blood sample was drawn after an overnight fasting period of at least eight hours.

Plasma total cholesterol, plasma triglycerides, and plasma glucose was determined using an automated enzymatic procedure on a Vitros 250 (Johnson & Johnson, Rochester, New York, USA). Low density lipid (LDL)- and high density lipid (HDL)- cholesterol were measured using a colorimetric assay on a Hitachi 904 (Johnson & Johnson, Rochester, New York, USA).

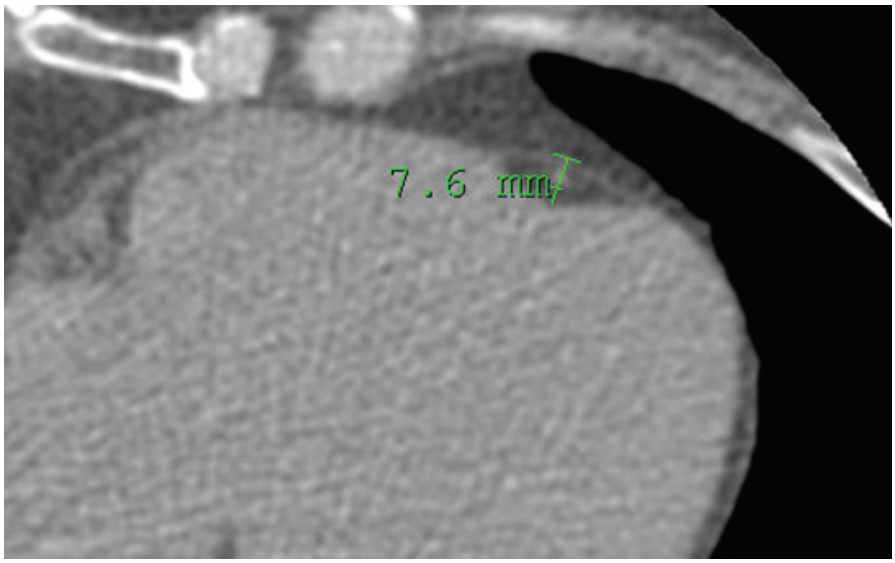

Figure 1. This multi-detector CT calcium scoring scan clearly shows the pericardium with underneath it the pericardial adipose tissue (EAT) surrounding the left anterior descending coronary artery (LAD). At this level the measurements of EAT in the LAD area can be done perpendicularly to the heart.

Cardiac imaging and calcium measurements

The amount of calcium in the coronary arteries was assessed with a multi detector-row CT (MDCT) scanner (Mx 8000 IDT 16, Philips Medical Systems, Best, The Netherlands). Subjects were positioned within the gantry of the MDCT scanner in a supine position. During a single breath-hold, images of the heart, from the level of the tracheal bifurcation to below the base of the heart, were acquired using prospective ECG triggering at 50-80% of the RR-interval, depending on the heart rate. Scan parameters were 16x1.5 mm collimation, 205 mm field of view (FOV), 0.42

s rotation time, 0.28 s scan time per table position, 120 kVp and 40 – 70 mAs (patient weight <70 kg: 40 mAs; 70-90 kg: 55 mAs; >90 kg: 70 mAs). Scan duration was approximately 10 seconds, depending on heart rate and patient size. From the acquired raw data, the whole volume was reconstructed with an intermediate reconstruction algorithm in non-overlapping data sets of 1.5 mm thick sections. Quantification of coronary calcium was performed on a separate workstation with software for calcium scoring (Heartbeat-CS, Extended Brilliance Workspace, Philips Medical Systems, Best, The Netherlands). All regions with a density over 130 Hounsfield units within the coronary arteries were manually identified as potential calcifications. To reduce the influence of noise, the minimum size of a calcified lesion was set at 0.5 mm^2. The peak density in Hounsfield units and the area in mm^2 of each selected region were calculated. The Agatston CAC score was obtained by multiplying the area by a weighting factor that is dependent on the peak signal anywhere in the lesion.[23] The scores of individual lesions were added to obtain the Agatston CAC score for the entire coronary tree. A reproducibility study in which a subgroup of 76 women were scanned twice revealed an intra-class correlation coefficient of 0.98 for the Agatston CAC score.[24]

Epicardial Adipose Tissue measurements

The amount of epicardial adipose tissue surrounding the coronary arteries was quantified on the MDCT scans, using a standardized method. Scans were loaded into a regular Philips CT application (Extended Brilliance Workspace, Philips Medical Systems, Best, The Netherlands). The researcher adjusted window settings to make the pericardium and epicardial adipose tissue visible. Next, the sections were determined where axial cuts are perpendicular to the surface of the heart (in order not to overestimate EAT diameter due to obliquity). This was done separately for the 3 main coronary arteries: right coronary artery (RCA), left anterior descending (LAD) coronary artery and the left circumflex (LCX) coronary artery. If more than one axial cut was perpendicular to the heart, the one with the most distinct layer of EAT was chosen for the measurements. At each of the three main coronary territories maximal EAT thickness (mm) was determined perpendicular to the pericardium (Figure 1). A number of 32 scans were evaluated a second time by a different observer in order to

determine inter-observer variability. This was regarded as sufficient to obtain an adequate estimate of reproducibility of the reading method.

One reader (CJR) read all the images. At the time of reading he was blinded to the study hypothesis and to the levels of risk factors in participants to limit the potential for bias. Furthermore, CAC measurements were performed by a different person (AR).

Data analysis

Baseline characteristics were expressed according to the quartile distribution of average peri-coronary EAT thickness. Reproducibility of the EAT measurements was assessed by estimation of the intra-class correlation coefficient and the 95% limits of agreement.[25]

The analyses were performed using the average thickness of EAT measurements of the RCA, the LAD and the LCX as continuous variable. Continuous variables were computed as mean and standard deviation and categorical variables were expressed as percentage.

In order to study whether the 10% random selection of the 5844 might have severely biased our findings, we compared the baseline characteristics of the 573 participating women with the characteristics of the 5844 women using regression models. Please note that (positive) selection bias occurs only when the selection process results in oversampling of subjects with both increased levels of exposure of interest and increased levels of the outcome. Extreme bias towards a null finding may occur when extreme oversampling occurs in both exposure and outcome.

First, the mean levels of all risk factors were studied across quartiles of the average EAT measurements. Since EAT was strongly related to age, all the relations with risk factors were initially adjusted for age using linear regression models. Risk factors that were related to EAT with $p<0.10$ were entered into a multivariable linear regression model to study the independent relationships. In case two factors interfered (for example weight and body mass index) one was left out.

Next, the relation between EAT and coronary calcification was assessed using linear regression models. Vascular risk factors were not added into these models, because we thought of traditional risk factors as intermediates in the process of EAT influencing the process of atherosclerosis. CAC was categorized as proposed by

Rumberger et al.[10]. Mean EAT thickness across categories of CAC were evaluated using an age-adjusted model. Model assumptions were checked graphically by qq plots of predicted values versus residuals. All statistical analyses were performed with the statistical package SPSS (SPSS for Windows, release 12.0.1, 2004: Chicago, Ill, SPSS Inc). Associations were considered significant at $p<0.05$. All statistical tests were 2 sided.

Results

The women were between 57 and 81 years of age (average 66.8±5.5). The average thickness of the layer of EAT surrounding the coronaries was 16.5±4.3 mm (range 5.9, 34.6 mm) in the RCA area, 6.4±2.2 mm (range 2.0, 14.0 mm) in the LAD area and 10.8±3.0 mm (range 2.8 to 29.1 mm) in the LCX area. Average thickness over all three territories was 11.2±2.2 mm (range 5.4, 19.1). According to visual inspection, average EAT thickness is normally distributed (Figure 2). The intra-class correlation coefficient was 0.76 (95% CI 0.50 to 0.88). According to Bland-Altman analysis the 95% limits of agreement for the average EAT thickness measurements were -4.0 and 7.1 mm. In 12 subjects CT scans could not be evaluated due to poor image quality. These persons were excluded from the analysis.

The baseline characteristics of the 573 participating women showed modestly lower levels of most vascular risk factors, and equal distribution of waist-to-hip ratio. Thus, in the participating women the CAC is expected to be lower as well as EAT since determinants of both show lower levels. Therefore, if anything, our relation is most likely an underestimate of the truth.

Table 1 describes the baseline characteristics of the study cohort according to quartiles of average peri-coronary EAT thickness. Women with the highest EAT values (forth quartile) were on average older, had a higher weight, a higher body mass index, a larger waist circumference and waist to hip ratio, had higher systolic blood pressure, more often used antihypertensive medication, a lower HDL levels and higher triglycerides, and a higher glucose level than women with the lowest EAT values (first quartile). Furthermore, CAC levels increased with increasing EAT (figure 3).

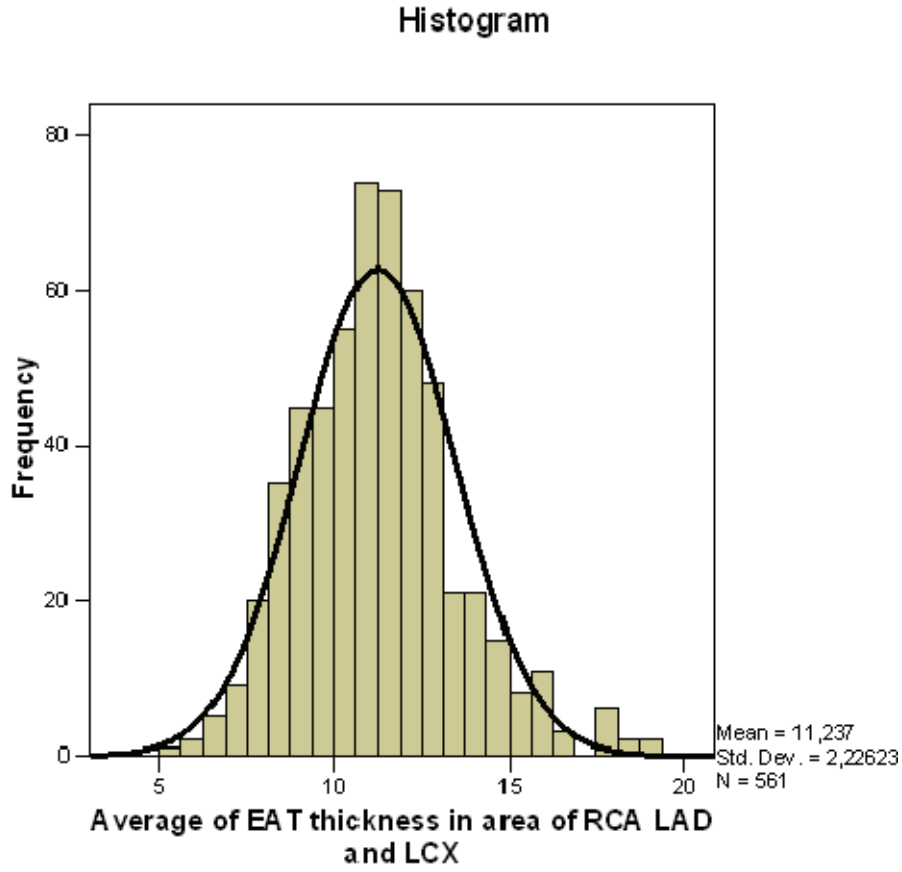

Figure 2. The diagram shown above displays the distribution of the epicardial adipose tissue thickness averaged over the three coronary arteries (RCA, LAD and RCX).

EAT was positively related to age (p=0.002). Table 2 provides results from age-adjusted linear regression analyses with average EAT thickness over the three coronary arteries. Significant positive relations were found for weight, body mass index, waist circumference, waist-to-hip ratio, systolic blood pressure, use of anti hypertensive drugs, triglycerides and glucose. HDL cholesterol was inversely related to the layer of peri-coronary EAT. When we entered those risk factors with $p<0.10$ into a multivariable linear regression model (table 3, model 1), age, weight, current smoking (borderline significant) and serum glucose were significantly related to the average thickness of EAT around the coronary arteries. As a measure for serum lipid status we took both HDL and triglycerides into the model. When, however, waist circumference entered the model (table 3, model 2) all traditional risk factors but age and waist circumference lost their significant relation with EAT.

Table 1. Characteristics of 573 Postmenopausal Women by EAT thickness quartiles.

	Quartiles of overall average EAT thickness				p
	1	2	3	4	
number of subjects	140	140	140	141	
age, years	65.6±5.2	67.3±4.9	66.9±5.6	67.4±6.0	
weight, kg	68.1±12.6	70.6±11.4	77.4±41.4	77.0±12.7	<0.001
height, m	1.64±0.06	1.65±0.06	1.65±0.06	1.65±0.06	0.598
Body Mass Index, kg/m2	25.1±4.3	26.1±4.0	27.2±3.8	28.5±4.9	<0.001
hip circumference, cm	99.1±8.2	103.4±38.5	102.2±7.2	103.5±8.0	0.095
waist circumference, cm	81.4±10.7	84.5±9.5	87.0±9.3	91.1±11.7	<0.001
waist to hip ratio	0.82±0.06	0.84±0.09	0.85±0.06	0.88±0.08	<0.001
systolic blood pressure, mm Hg	131.8±19.1	136.3±20.3	139.8±23.4	137.1±18.7	0.034
diastolic blood pressure, mm Hg	71.1±9.5	72.5±9.6	72.8±9.7	72.2±8.4	0.079
use of BP lowering drugs	21.4%	17.9%	25.9%	32.6%	0.007
hypertension 140/90 or medication	47.1%	45.7%	54.3%	55.3%	0.096
total cholesterol, mmol/l	5.9±0.9	6.1±0.9	6.1±1.1	6.0±1.1	0.687
HDl cholesterol, mmol/l	1.4±0.4	1.4±0.4	1.4±0.3	1.3±0.4	0.005
LDL cholesterol, mmol/l	4.1±0.8	4.2±0.8	4.3±1.0	4.2±0.9	0.339
triglycerides, mmol/l	1.1±0.5	1.2±0.6	1.3±0.6	1.3±0.7	0.001
current smoking	8.6%	12.1%	12.1%	13.5%	0.088
former smoking	47.1%	44.3%	42.1%	45.4%	0.545
previous CVD	2.9%	2.9%	5.0%	3.5%	0.707
diabetes mellitus	5.7%	5.0%	2.9%	7.1%	0.200
glucose, mmol/l	5.5±0.8	5.6±0.9	5.5±0.6	5.8±1.3	<0.001
CAC score, Agatston	58.8±151.3	116.3 ±269.0	132.8±331.3	152.2±315.9	0.026

Continuous variables are presented as mean±SD. The last column shows significance in age-adjusted linear regression analysis with average EAT thickness of the three coronary arteries as dependent variable. The cut points of the quartiles are 9.7, 11.2 and 12.5 mm.

The Spearman correlation between EAT and waist circumference was 0.34 (p<0.001). A correlation of 0.34 does not indicate severe collinearity.

Figure 3 depicts the univariable relation between EAT and CAC. The Spearman correlation between EAT and CAC was 0.016 (p=0.003). A graded association was seen: the higher the amount of EAT, the higher the amount of CAC. This relationship remained statistically significant when adjusted for age in a linear regression model (p=0.026). The relation of EAT and CAC was not significant, when stratified according to waist circumference. Age-adjusted regression analyses showed that the diameter of EAT around the LAD and the RCA was strongly

correlated to the presence of coronary calcification in the same artery (p=0.034 and p=0.03, respectively). This was not found for the RCX (p=0.285).
Additional analyses, excluding participants with previous cardiovascular disease (acute myocardial infarction, coronary artery stenosis, carotid artery stenosis, and stroke) did not materially change the results.

Table 2. Age-adjusted relations between EAT and risk factors.

Cardiovascular Risk Factor	Beta	95% Confidence Interval for Beta		p	R^2
		Lower Bound	Upper Bound		
weight, kg	0.017	0.010	0.025	<0.001	0.05
height, m	0,821	-2.234	3.876	0.598	0.02
Body Mass Index, kg/m2	0.161	0.121	0.200	<0.001	0.12
hip circumference, cm	0.008	-0.001	0.017	0.095	0.02
waist circumference, cm	0.076	0.060	0.092	<0.001	0.15
waist to hip ratio	9.699	7.380	12.018	<0.001	0.12
systolic blood pressure, mm Hg	0.010	0.001	0.019	0.034	0.02
diastolic blood pressure, mm Hg	0.018	-0.002	0.037	0.079	0.02
use of BP lowering drugs	0.592	0.165	1.019	0.007	0.03
hypertension 140/90 or medication	0.317	-0.057	0.691	0.096	0.02
total cholesterol, mmol/l	0.038	-0.147	0.223	0.687	0.02
HDl cholesterol, mmol/l	-0.749	-1.267	-0.231	0.005	0.03
LDL cholesterol, mmol/l	0.098	-0.103	0.300	0.339	0.02
triglycerides, mmol/l	0.502	0.206	0.798	0.001	0.04
current smoking	0.500	-0.074	1.074	0.088	0.02
former smoking	-0.114	-0.482	0.255	0.545	0.02
previous CVD	0.189	-0.801	1.180	0.707	0.02
diabetes mellitus	0.540	-0.287	1.368	0.200	0.02
glucose, mmol/l	0.349	0.153	0.544	<0.001	0.04
CAC score, Agatston	0.161	0.019	0.303	0.026	0.02

Results are presented as linear regression coefficients with corresponding 95% confidence limits, p values and the amount of variance explained (R^2). Beta means a change in EAT (mm) with an increase of one unit in the risk factor. For example, change of 1 kg in weight leads to a change in EAT of 0.017 mm.

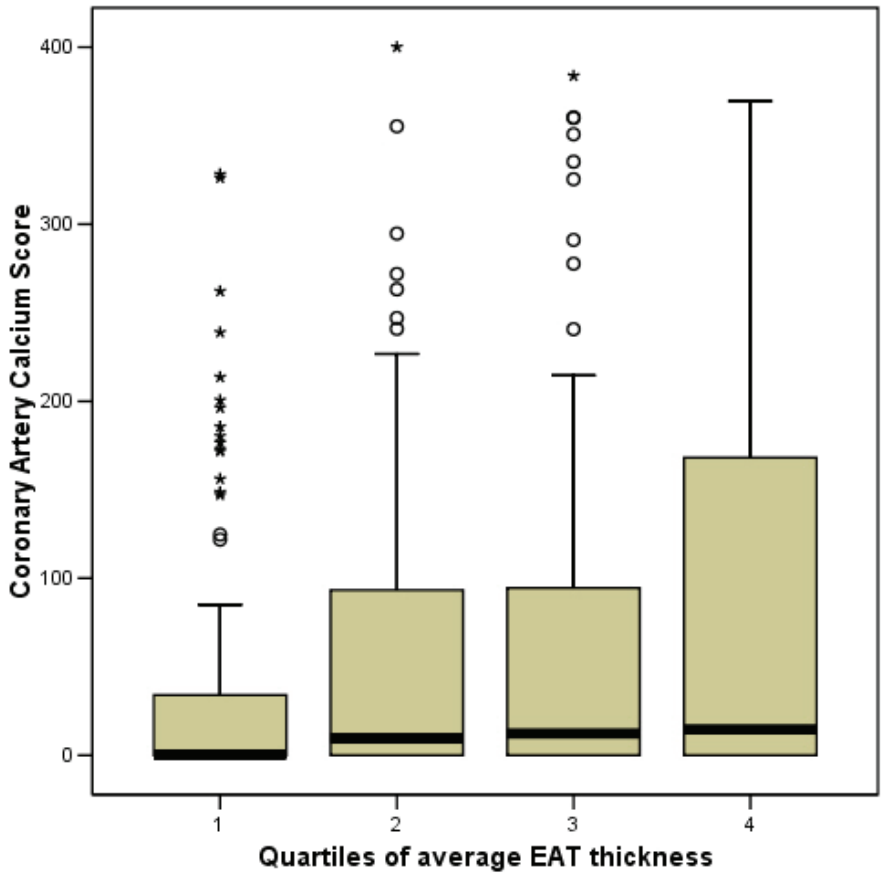

Figure 3. Coronary artery calcification (CAC) scores by pericoronary EAT quartile.

Table 3. Multi-variable relations between EAT and risk factors.

	Beta	95% Confidence Interval for B		p
		Lower Bound	Upper Bound	
Model 1				
age	0.047	0.013	0.081	0.007
weight	0.014	0.006	0.022	0.001
hip	0.002	-0.007	0.011	0.674
systolic blood pressure	0.007	-0.002	0.016	0.142
high density lipoprotein	-0.254	-0.838	0.330	0.393
triglycerides	0.229	-0.114	0.573	0.191
current smoking	0.530	-0.045	1.105	0.071
glucose	0.213	0.011	0.415	0.039
Model 2				
age	0.039	0.006	0.072	0.020
weight	0.000	-0.009	0.008	0.966
hip	-0.004	-0.013	0.005	0.344
systolic blood pressure	0.003	-0.006	0.012	0.501
high density lipoprotein	0.070	-0.497	0.637	0.809
triglycerides	0.051	-0.282	0.385	0.762
current smoking	0.419	-0.133	0.972	0.137
glucose	0.036	-0.164	0.236	0.725
waist	0.075	0.054	0.096	<0.001

Results are presented as linear multivariable regression coefficients with corresponding 95% confidence limits. Beta means a change in EAT (mm) with an increase of one unit in the risk factor, while keeping the other risk factors constant. Model 1 describes multivariable regression of the risk factors which were related to EAT with $p<0.10$ in the age-adjusted regression models (see table 2). In case two factors interfered (for example weight and body mass index) one was left out. Model 2 is the same as model 1, but after addition of waist circumference. Risk factors that were significantly related to EAT in model 1 (age, weight, current smoking (borderline significant), glucose) lost their significance (except for age) in model 2 due to the addition of waist circumference (highly significant) in the model. The R^2 of model 1 is 8.3% and the R^2 of model 2 is 15.8%.

Discussion

The major findings of this population-based study in healthy post-menopausal women are that EAT directly surrounding the coronary arteries is related to a large number of vascular risk factors. Most of these relations are attenuated when an adjustment was made for waist circumference, suggesting that EAT and abdominal adipose tissue are strongly related. Furthermore, peri-coronary EAT is related to CAC, suggesting a role in the development of coronary atherosclerosis.

Previous studies on EAT thickness were mainly based on echocardiography and have measured EAT thickness on the right ventricle only[1;17;18;20] or combined epicardial and pericardial adipose tissue surrounding the heart.[21] Differentiation between epi- and pericardial fat may be difficult at echocardiography.[26] On CT the pericardium is readily identified, resulting in easy differentiation between epi- and pericardial fat. In contrast to previous studies we focused on epicardial fat surrounding the arteries because of the notion that it is the local fat that may drive the development of atherosclerosis. Sample size of the echocardiography studies ranged from 22 to 72 persons. Moreover, studied populations differed from our general population sample.[18;20;21] The studies using echocardiography to measure EAT thickness on the right ventricle showed relations with waist circumference, diastolic blood pressure and left ventricular mass.[1;17;18] Our findings for peri-coronary EAT are in agreement with this. We report a strong relation between the peri-coronary EAT and age. Although autopsy studies have found a relationship between EAT and age[27], many imaging studies have not been able to confirm this.[1;17;20] The fact that we found more epicardial adipose tissue in the area of the right coronary artery is in concordance with literature.[27;28] The relation between (peri-coronary) EAT and coronary calcification has not been reported before. To the best of our knowledge, the present study is first to demonstrate an age-adjusted relation between EAT and subclinical coronary atherosclerosis. This effect of peri-coronary EAT could be direct rather than systemic, because of the endocrine characteristics of this adipose tissue[9] and its direct location near the coronary vessel wall, without any fascia preventing paracrinologic migration of bio-active molecules. Experimental studies showed the possibility of this inward direction of inflammatory mediators.[13-15] Animal studies indicated that the rate of fatty acid release by EAT is approximately twice that of the pericardial and peri-renal depots[29] and in humans low levels of plasma adiponectin, a protein secreted by adipose tissue, is associated with increased cardiovascular risk.[30;31] This adiponectin is directly expressed by EAT and it is expressed in lower quantities in EAT from patients with coronary artery disease.[32]

Some limitations of the study need to be addressed. We did not collect either CT or ultrasound measurements[33] on abdominal adipose fat, which would have made our study results stronger in elucidating the relation between EAT and abdominal adipose tissue. Also, it is a cross-sectional study, which limits conclusions

on cause and consequence. Although our results are based on data obtained in women, we do not know any mechanistic reason why these findings may not also apply to men. Nevertheless, confirmation of these findings in studies among men is recommended to firmly support that notion. The strength of the study, however, is its population-based nature and its fairly large sample size. CT offers excellent spatial resolution of approximately 0.4-0.6mm, which is superior to competing imaging modalities (figure 1).[26]

The present study is not able to distinguish between peri-coronary EAT having a direct effect on the development of CAC or peri-coronary EAT being merely a reflection of systemic elevated risk factors that lead to the development of CAC. Future studies on this issue are needed, as are studies relating the presence of peri-coronary EAT to the occurrence of cardiovascular events.

In conclusion, our findings in a population based study of CT scans in post-menopausal women provide evidence that the layer of EAT directly surrounding the coronary arteries is strongly associated with several vascular risk factors and coronary artery calcification.

Acknowledgements

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Chapter 8

Metabolic syndrome and peri-coronary epicardial adipose tissue in healthy post-menopausal women

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Submitted

Abstract

Objective: Epicardial Adipose Tissue (EAT) is a layer of visceral fat between the myocardium and the pericardium and has a similar origin as abdominal fat. Recent reports relate peri-coronary EAT to cardiovascular risk factors and coronary atherosclerosis. The metabolic syndrome is associated with increased cardiovascular risk. The aim of this study was to determine whether the metabolic syndrome is related to peri-coronary EAT.

Design and methods: We performed a cross-sectional study among 573 healthy post-menopausal women. Detailed information on vascular risk factors was obtained. Metabolic syndrome was assessed using the National Cholesterol Education Program Adult Treatment Panel III (NCEP) definition. EAT was determined on cardiac CT scans in the areas of right (RCA), left anterior descending (LAD) and circumflex coronary artery (LCX). At each of these sites EAT area was measured on transverse sections. Linear and logistic regression models were used to assess the relations.

Results: Women were between 57 and 81 years of age (mean 67±5). Mean EAT area was 236.8±94.9 mm^2 (range 66.7, 1152.8). In 39.5% of women the metabolic syndrome was present. EAT was positively related to age (p=0.01). In age-adjusted logistic regression models the risk of the metabolic syndrome being present was much higher in those in the upper quartile of EAT distribution compared to the lowest quartile (OR 4.20; 95% CI 2.50;7.03). Furthermore, there was a graded relation between the number of metabolic syndrome factors and peri-coronary EAT. The relation persisted in lean subjects, i.e. those with a waist circumference below the median (OR 2.56; 95% CI 1.04;6.25).

Conclusions In post-menopausal women the metabolic syndrome is related to peri-coronary EAT. This relation persisted in lean subjects.

Introduction

Epicardial Adipose Tissue (EAT) is a layer of visceral fat between the myocardium and the pericardium. EAT, as well as intra-abdominal fat, appears to be a rich source of bioactive molecules [1]. Coronary atherosclerosis is considered an excessive inflammatory and proliferative process inside the vascular wall [2;3]. The secretion of inflammatory mediators from the adipose tissue surrounding the epicardial coronary arteries may have an important role in the development of coronary atherosclerosis [4-9]. It is therefore conceivable that EAT contributes to the development of atherosclerosis 'from outside to inside'[10]. Indeed, several studies have indicated an association between EAT and CAD [11-14] and recently we reported the relation between peri-coronary EAT and coronary artery calcification (CAC) [9]. In addition, common cardiovascular risk factors have been associated with EAT [15-17]. Waist circumference, as an indicator of visceral fat, may be the strongest factor related to EAT [9]. Visceral fat has been shown to be an important indicator of cardiovascular risk [16;18-21], and visceral fat is an important determinant of the metabolic syndrome. The metabolic syndrome, as being a combination of unfavorable levels of risk factors, consisting of hypertension, high fasting plasma glucose, low HDL cholesterol and increased waist circumference [22] substantially increases risk of developing coronary artery disease (CAD) [23-26]. However, it is unclear whether the metabolic syndrome is associated with an increased prevalence of peri-coronary EAT. The aim of this study was to investigate the relation between peri-coronary EAT and the metabolic syndrome and whether this relation was modified by waist circumference.

Design and methods

Study population

We used data from a cross-sectional study among 573 healthy post-menopausal women as described earlier [27]. Between October 2002 and December 2004, 1000 women were randomly selected from 5844 participants of the PROSPECT study who were post-menopausal and did not use contraceptives or hormone replacement therapy. The PROSPECT study is one of the two Dutch cohorts participating in the European Prospective Investigation into Cancer and Nutrition (EPIC)[28]. Of these 1000 women, a random selection of 573 underwent a multi-detector CT (MDCT)

examination during a single visit. The Medical Ethical Committee of the University Medical Center Utrecht approved the study and written informed consent was obtained from all participants. The study complies with the Declaration of Helsinki, the Medical Ethical Committee of the University Medical Center Utrecht approved the study and written informed consent was obtained from all participants. Current cardiovascular drug use (blood pressure lowering, lipid lowering and glucose lowering drugs) was assessed by asking women to bring all packages to the study center. Smoking behavior, medical history and cardiovascular family history were assessed by a questionnaire. Height and weight were measured and body mass index was calculated as weight divided by height squared (kg/m2). Waist-to-hip ratio was assessed. Systolic and diastolic blood pressures were measured at both arms with an automated and calibrated blood pressure device (DINAMAP XL, Critikon, Johnson & Johnson, Tampa, Florida, USA) with the subject in supine position. A venous blood sample was drawn after an overnight fasting period of at least eight hours. Plasma total cholesterol, plasma triglycerides, and plasma glucose was determined using an automated enzymatic procedure on a Vitros 250 (Johnson & Johnson, Rochester, New York, USA). Low density lipid (LDL)- and high density lipid (HDL)- cholesterol were measured using a colorimetric assay on a Hitachi 904 (Johnson & Johnson, Rochester, New York, USA). Diabetes mellitus was defined as fasting blood glucose greater than 6.9 mmol/L and/or the use of antidiabetic medication.

Cardiac imaging

Participants underwent cardiac imaging with a MDCT scanner (Mx 8000 IDT 16, Philips Medical Systems, Best, the Netherlands) for the purpose of CAC scoring. Subjects were positioned within the gantry of the MDCT scanner in a supine position. During a single breath-hold, images of the heart, from the level of the tracheal bifurcation to below the base of the heart, were acquired using prospective ECG triggering at 50-80% of the RR-interval, depending on the heart rate. Scan parameters were 16x1.5 mm collimation, 205 mm field of view (FOV), 0.42 s rotation time, 0.28 s scan time per table position, 120 kVp and 40 – 70 mAs (patient weight <70 kg: 40 mAs; 70-90 kg: 55 mAs; >90 kg: 70 mAs). Scan duration was approximately 10 seconds, depending on heart rate and patient size. From the acquired raw data, the whole volume was reconstructed with an intermediate reconstruction algorithm in

non-overlapping data sets of 1.5 mm thick sections. Detailed scan parameters and procedures are described elsewhere [29].

Epicardial Adipose Tissue measurements

The amount of peri-coronary epicardial adipose tissue was quantified on the MDCT scans with a standardized method. Scans were loaded into a regular Philips CT application (Extended Brilliance Workspace, Philips Medical Systems, Best, the Netherlands). The researcher adjusted window settings to make the pericardium and epicardial adipose tissue visible. Next, the sections were determined where axial cuts are perpendicular to the surface of the heart (in order not to overestimate EAT diameter due to obliquity). This was done separately for the 3 main coronary arteries: right coronary artery (RCA), left anterior descending (LAD) coronary artery and the left circumflex (LCX) coronary artery. If more than one axial cut was perpendicular to the heart, the one with the most distinct layer of EAT was chosen for the measurements. At each of the three main coronary territories the maximal EAT area (mm2) was determined perpendicular to the pericardium (Figure 1). One reader read all the images. A number of 32 scans were evaluated a second time by a different reader in order to determine inter-observer variability. This was seen as sufficient to obtain an adequate estimate of reproducibility of the reading method.

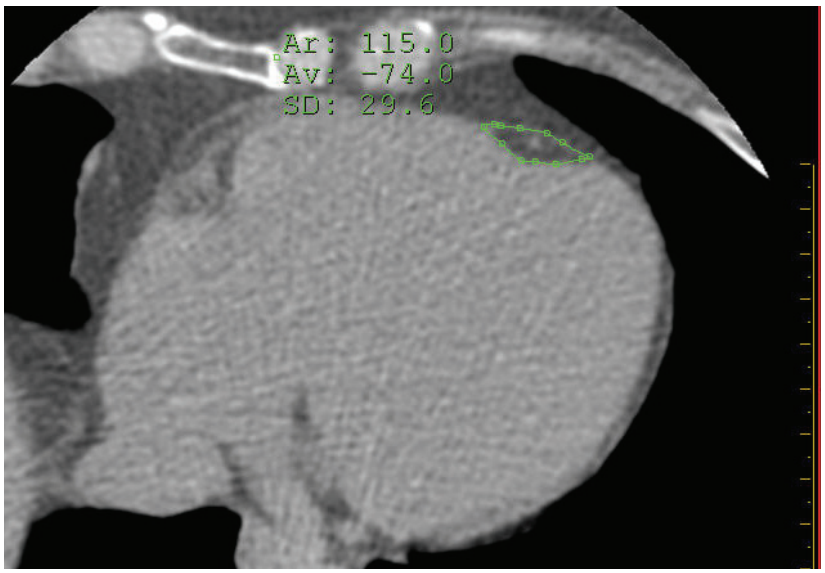

Figure 1. Multi-detector CT of the heart. The above CT calcium scoring scan shows the pericardium with underneath it the epicardial adipose tissue (EAT) surrounding the left anterior descending coronary artery (LAD). At this level the area measurements of EAT in the LAD area can be done perpendicularly to the heart.

Definition of metabolic syndrome

The metabolic syndrome was defined according to the National Cholesterol Education Program (NCEP)/Third Adult Treatment Panel (ATPIII) criteria [22], as modified by the NHLBI [30]. Subjects with 3 or more of the following criteria met the definition for metabolic syndrome: abdominal obesity (waist circumference >88 cm (=35 inches) in women), high blood pressure (≥130 mm Hg systolic or ≥85 mm Hg diastolic or antihypertensive drug treatment), hypertriglyceridemia (serum triglycerides ≥1.70 mmol/L (=150mg/dl)), low HDL-cholesterol (serum HDL-cholesterol <1.29 mmol/L (=50mg/dl)), high fasting glucose (fasting glucose ≥5.6 mmol/L (=100mg/dl) or drug treatment for elevated glucose).

Data analysis

General characteristics are given as means with corresponding standard deviations or as proportions. In 5 subjects CT scans could not be evaluated due to poor image quality. These scans were excluded from the analyses. The primary analyses were performed using the average area of EAT around the RCA, the LAD and the LCX. The relation between quartiles of EAT and the metabolic syndrome was studied in age adjusted logistic regression models. Analyses were repeated in strata of median waist circumference (cut-off 86cm=33.8 inches). All statistical analyses were performed with the statistical package SPSS (SPSS for Windows, release 14, 2006: Chicago, Ill, SPSS Inc). Associations were considered significant at $p<0.05$.

Results

The women were between 57 and 81 years of age (mean 66.8±5.5). Spearman correlation coefficient for interobserver variability in EAT measurements was 0.75 and the Intraclass correlation coefficient was 0.66. Average EAT area was 336.9±170.2 mm^2 (range 90.7, 3207.0) for the RCA area, 182.6±122.7 mm^2 (range 30.7, 908.4) for the LAD area and 192.4±80.5 mm (range 29.7, 519.9) for the LCX. Overall average EAT area was 237.5±94.8 mm^2 (range 66.7, 1152.8). Peri-coronary EAT was categorized into quartiles: low (less than 180 mm^2), moderate (between 180 and 223 mm^2), high (between 223 and 275 mm^2) and very high (more than 275 mm^2). In 39.5 % of the participants the metabolic syndrome was present. (Table 1)

Table 1. General characteristics of the study population (n=564). Data are presented as mean + standard deviation.

	Mean	Std. Deviation
Age (yrs)	66.8	5.5
Weight (kg)	73.2	23.5
Height (m)	1.65	0.06
Body mass index (kg/m²)	26.7	4.4
Hip circumference (cm)	102.0	20.3
Waist circumference (cm)	86.0	10.9
Waist to hip ratio	.85	0.06
Systolic blood pressure (mmHg)	136	20
Diastolic blood pressure (mmHg)	72	9
Use of blood pressure lowering drugs (%)	24.0	
Total cholesterol (mmol/l)	6.0	1.0
HDl cholesterol (mmol/l)	1,4	0.4
LDL cholesterol (mmol/l)	4.2	0.9
Triglycerides (mmol/l)	1.2	0.6
Current smoking (%)	11.5	
Former smoking (%)	44.7	
Previous cardiovascular disease (%)	3.5	
Fasting glucose (mmol/l)	5,6	0.9
Average peri-coronary EAT area (mm²)	237.5	94.8
Metabolic syndrome (%)	39.5	
Metabolic syndrome factor hypertension (%)	66.1	
Metabolic syndrome factor glucose intolerance (%)	15.2	
Metabolic syndrome factor HDL (%)	47.9	
Metabolic syndrome factor triglycerides (%)	17.8	
Metabolic syndrome factor waist circumference (%)	39.9	

Abbreviations: HDL = high density lipoprotein; LDL = low density lipoprotein; EAT = epicardial adipose tissue.

The EAT areas were much larger among women with the metabolic syndrome with a mean difference of 44.5 mm² (95%CI 28.8;60.2). Age-adjusted logistic regression model showed a gradual increased risk of the metabolic syndrome being present with increasing EAT levels. The odds of the metabolic syndrome being present were 4.2 (95% CI 2.5; 7.0) times higher in those in the upper quartile of EAT distribution compared to the lowest quartile (table 2). Additional adjustment for LDL-cholesterol, lipid lowering drugs and smoking did not materially alter these findings. In addition, the mean EAT area increased gradually with increasing number of metabolic syndrome components (figure 2).

Median waist circumference was 86.0±10.9 cm. The relation between peri-coronary EAT and the metabolic syndrome persisted among women with low waist circumference (OR 2.56; 95%CI 1.04;6.25). In contrast, no relation was found among women with a high circumference (OR 1.37; 95%CI 0.59;3.18).

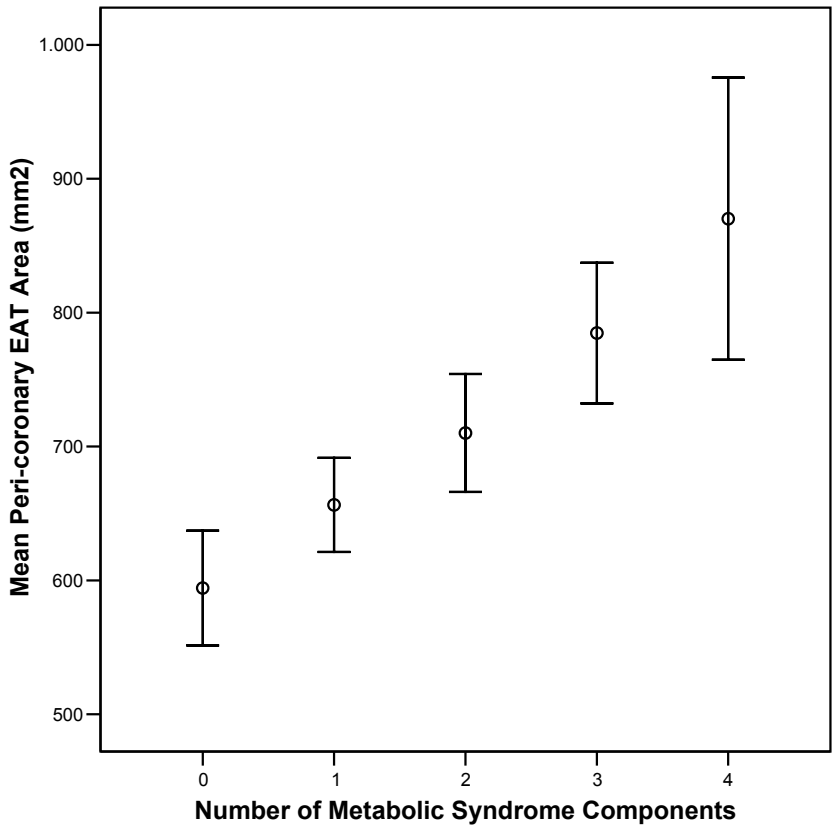

Figure 2. The mean peri-coronary EAT area increased with the number of metabolic syndrome components. Because of the relatively small number of patients who had 4 or 5 components, the latter groups were combined. Values are crude means with corresponding 95% confidence intervals.

Table 2. The relation between peri-coronary epicardial adipose tissue and the metabolic syndrome risk factors.

	EAT quartile groups			
	1 (low)	**2 (moderate)**	**3 (high)**	**4 (very high)**
	reference	**OR* (95%CI)**	**OR* (95%CI)**	**OR* (95%CI)**
Metabolic syndrome	1	1.90 (1.13-3.19)	2.13 (1.27-3.59)	4.20 (2.50-7.03)
Elevated triglycerides ≥1.7 mmol/l (150 mg/dl)	1	1.23 (0.65-2.32)	0.94 (0.48-1.82)	1.74 (0.94-3.19)
Reduced HDL <1.3 mmol/l (50 mg/dl)	1	1.17 (0.73-1.88)	1.73 (1.07-2.80)	1.72 (1.07-2.77)
Hypertension ≥130/≥85 mmHg (or on antihypertensive drug treatment)	1	1.40 (0.85-2.29)	1.53 (0.92-2.54)	1.84 (1.10-3.08)
Elevated fasting glucose ≥5.6 mmol/l (100 mg/dl) or on drug treatment for elevated glucose	1	1.80 (1.10-2.94)	1.49 (0.90-2.45)	2.00 (1.22-3.27)
Elevated waist circumference ≥88 cm (≥35 inches)	1	2.81 (1.60-4.95)	3.85 (2.20-6.74)	9.02 (5.12-15.89)

The study population was divided into quartiles of peri-coronary EAT (see text). For each quartile the odds ratio (OR) (95% confidence interval) is presented for the presence of the factors listed. Abbreviations: HDL = high density lipoprotein.
*Adjusted for age.

Discussion

In the current population based study we have demonstrated that in healthy post-menopausal women the metabolic syndrome is related to the presence of peri-coronary EAT. Furthermore, a graded relation was found between the number of metabolic syndrome components and peri-coronary EAT. The strongest metabolic syndrome component in this relation seemed to be waist circumference. Yet, in patients with lower than median waist circumference (<86cm or 33.8 inches) the relation of peri-coronary EAT with the metabolic syndrome persisted. This finding may suggest that fat around the coronary arteries is more than just a marker of abdominal visceral adiposity.

The metabolic syndrome may be related to atherosclerosis because of the fact that obese persons are prone to be insulin resistant [31]. Insulin resistance is associated with high levels of circulating adipocytokines. These adipocytokines are bioactive molecules that are secreted from visceral adipose tissue and modulate insulin resistance [32]. Circulating levels of adipocytokines were recently found to be associated with coronary atherosclerosis [33]. Our findings might point towards an alternative, complementary mechanism to explain the relation between metabolic syndrome and increased vascular risk. The metabolic syndrome may comprise increased risk of CAD influenced by the presence of peri-coronary EAT [34], because EAT is a rich source of pro-inflammatory adipocytokines [1]. Studies demonstrate that peri-adventitial application of bioactive molecules (e.g. endotoxins, chemoattractant protein-1 (MCP-1), interleukin-1beta, or oxidized LDL) induce inflammatory cell influx into the arterial wall, coronary vasospasm, or intimal lesions, which suggests that these molecules may alter arterial homeostasis and may play a role in the process of atherosclerosis [1;5;6]. Although EAT is located in direct proximity to the epicardial coronary arteries, obese persons have more total visceral fat and therefore higher concentrations of circulating inflammatory cytokines and for this reason the role of adipocytokines secreted by the peri-coronary EAT may become relatively less important in the process of atherosclerosis [35]. Our findings that peri-coronary EAT was only associated with the metabolic syndrome in lean persons is in line with previous findings [11;35].

The metabolic syndrome received much criticism in recent years, because of uncertainty regarding its pathogenesis [36]. Its most important value, however, may be

its promise to identify asymptomatic individuals who are at increased risk of developing CAD [37]. EAT may fulfill at least a similar role, but may turn out to be a risk factor for the development of cardiovascular events. We will not know this until results from large prospective studies relating EAT to future risk of disease are available.

Our study has several limitations. The EAT measurement has some measurement error. Since this most likely leads to random misclassification, the magnitude of our findings are probably underestimations of the true relations. Secondly, our population sample of post-menopausal women is relatively small which limits the precision of the estimates. In addition, the data are of cross-sectional nature which limits causal and time frame related conclusions.

In conclusion, in a cohort of post-menopausal women the metabolic syndrome is strongly related to the presence of peri-coronary EAT, especially in the non-obese. Confirmation in future prospective research is recommended as well as studies into the role of peri-coronary EAT as a risk factor for the occurrence of cardiovascular events.

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Chapter 9

Epicardial adipose tissue and coronary artery disease in patients scheduled for coronary angiography

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Abstract

Fat surrounding coronary arteries might aggravate coronary artery disease (CAD). Aim was to investigate the relationship between epicardial adipose tissue (EAT) and peri-coronary fat and coronary atherosclerosis and coronary artery calcium (CAC) in patients suspected of CAD, and whether this relationship is modified by total body weight. Cross-sectional study in 128 patients with angina pectoris (61±6 years) undergoing coronary angiography. EAT volume and peri-coronary fat thickness were measured with cardiac computed tomography (CT). Severity of coronary atherosclerosis was assessed by the number of stenotic (≥50%) coronary vessels; extent of CAC was determined by Agatston score. Patients were stratified for median total body weight (body mass index (BMI) 27 kg/m^2). Overall, EAT and peri-coronary fat were not associated with severity of coronary atherosclerosis and extent of CAC. In patients with low BMI, those with multi-vessel disease had higher EAT volume (99.7 vs. 66.5 cm^3, p-value=0.04) and peri-coronary fat thickness (9.8 vs. 8.4 mm, p-value=0.06) compared to those without CAD. Also, patients with severe CAC had more EAT volume (108.0 vs. 68.7 cm^3, p-value=0.02) and peri-coronary fat thickness (10.0 vs. 8.2 mm, p-value=0.01) compared to those with minimal/absent CAC. In conclusion, EAT and peri-coronary fat were not associated with severity of coronary atherosclerosis and CAC in patients suspected of CAD. However, in those with low BMI, high EAT and peri-coronary fat are related to more severe coronary atherosclerosis and CAC. Fat surrounding coronary arteries may be involved in the process of coronary atherosclerosis, although this is different for patients with low and high BMI.

Introduction

Abdominal fat is able to produce large quantities of tumor necrosis factor-alpha, interleukin-6, free fatty acids and plasminogen activator inhibitor-1, all involved in accelerating atherosclerosis, plaque instability and arterial thrombosis.[1-3] Adipose tissue surrounding coronary arteries is in close contact with the adventitia and may aggravate vessel wall inflammation and stimulate the progression of atherosclerosis from 'outside to inside.[4;5] As body mass index (BMI) and abdominal fat are strongly associated with epicardial adipose tissue (EAT) [6;7] and are important determinants for the development of CAD[8;9] the influence of fat mass around the heart on the development of CAD may differ in patients with low and high BMI.[10]

Previously, the association between EAT thickness and CAD has been evaluated using echocardiography.[7;11;12] However, computed tomography (CT) is more accurate to quantify adipose tissue accumulation due to its higher spatial resolution.[13] The aim of the present study is to investigate the relation between EAT volume and peri-coronary fat thickness, quantified using cardiac CT, and the severity of coronary atherosclerosis and extent of coronary artery calcium (CAC) in patients suspected of CAD referred for coronary angiography, and whether this relationship is modified by body weight.

Methods

Patients originated from a diagnostic study at the University Medical Center Utrecht designed to establish the diagnostic accuracy of multi-slice computed tomography coronary angiography compared to conventional coronary angiography in the detection of significant coronary obstruction. Patients (n = 128) were referred for diagnostic coronary angiography or percutaneous coronary intervention because of stable angina pectoris (n = 100) or unstable angina pectoris (n = 28)[14;15] as clinically indicated at the discretion of the referring cardiologist. Subjects were divided by median BMI (cut off value: 27 kg/m2) into patients with low and high total body weight (BMI). The inclusion criteria were: age 50-70 years, and stable sinus rhythm. Patients with previous percutaneous transluminal coronary angioplasty or coronary artery bypass graft, serum creatinine levels >140 μmol/l, or known iodine-based contrast allergy were not included. CT coronary angiography was performed in all

patients within a month before or after conventional coronary angiography. The Medical Ethics Committee approved the study and all participants gave their written informed consent.

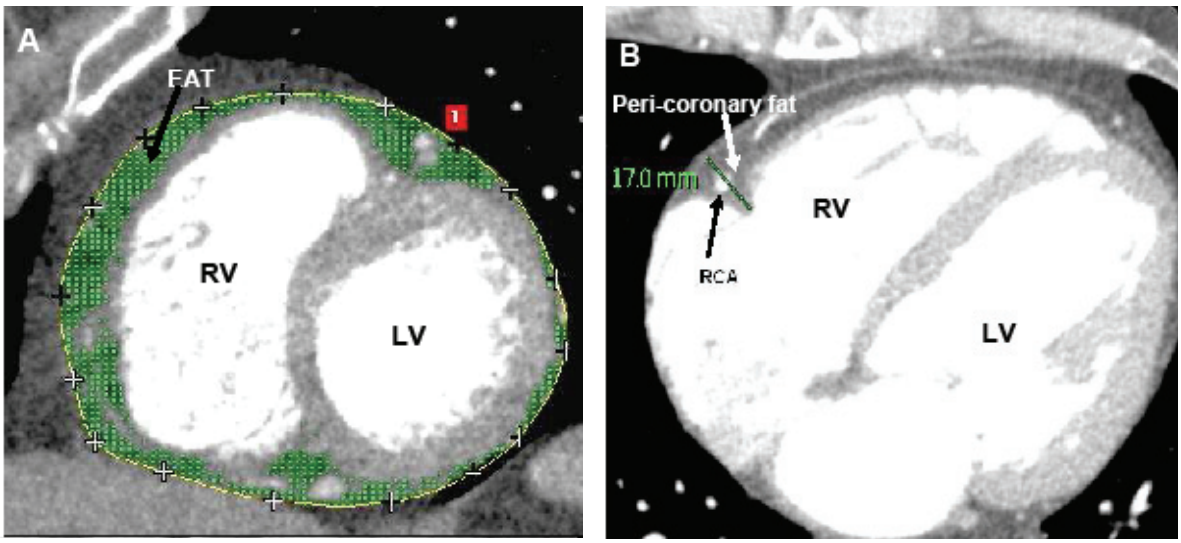

Figure 1. Epicardial adipose tissue (EAT) and peri-coronary fat measurements. (A) Region of interest (ROI) (yellow) on a cross-sectional image to determine the EAT area (green). EAT volume is the sum of the EAT areas on cross-sectional images. (B) Peri-coronary fat thickness (green).

Clinical information was obtained using a standardized health questionnaire. Height, body weight, and blood pressure were measured. Fasting blood was sampled to determine lipid, glucose and creatinine levels. Metabolic syndrome was diagnosed according to the Adult Treatment Panel III criteria as the presence of at least 3 or more metabolic abnormalities.[16] Because waist circumference was not available, a BMI of ≥30 kg/m² was used as determinant for obesity.[17]

Pericardial fat includes EAT (or peri-coronary fat) and paracardial fat which are separated by the fibrous pericardium. EAT and peri-coronary fat were quantified on electrocardiogram-gated diagnostic cardiac CT scans. CT studies were performed on a 64 detector-row CT scanner (Brilliance 64, Philips Medical Systems, Cleveland, OH, USA). Scan duration time was 7-10 seconds. Standard coronary imaging protocols were applied including the use of intra-venous beta-blockers for patients with heart rates >65 beats/min (unless contraindicated) and image acquisitions were performed during a breath-hold in inspiration. Imaging parameters were slice

collimation of 64 x 0.625 mm, gantry rotation time of 420 ms, tube voltage of 120 kV, tube current of 900 mAs. Contrast agent used was iopromide (Schering AG, Berlin, Germany), which was injected intravenously (1.6-2.0 g iodine/s depending on patient's body weight).

Measurements were performed in the most motionless phase of the cardiac cycle, which was most frequently a mid-diastolic phase, with retrospective cardiac gating at 70-80% of the R-R interval. The window settings were adjusted to properly visualize the adipose tissue and the pericardium.

EAT volume was defined as the total amount of adipose tissue between the surface of the heart and the visceral layer of the pericardium (visceral epicardium). EAT volume (cm3) was measured using two dedicated software programs (Extended Brilliance Workspace and Easy Vision, Philips Medical Systems, Best, The Netherlands). Measurements were performed on short-axis views of 2-mm-slice-thickness and 3-mm-intersection gaps. The stack of the short-axis views started at the apex just below the fibrous pericardium and extended until the center of the left atrium. An EAT area measurement was performed by tracing a single region of interest containing heart and EAT (Figure 1A) on the section obtained at each level. Regions of interest were placed at the visceral epicardium to exclude pericardial fluid. A density range between -200 and -30 Hounsfield Units was used to isolate the adipose tissue. Subsequently, the computer software program produced an accurate volume of EAT by adding up the EAT areas of 25-30 sections and taking into account slice thickness and intersection gap. The intra- and interobserver coefficient of variations, measured for 60 CT scans, were 3.1% and 5.0%, respectively.[18]

Peri-coronary fat was defined as the adipose tissue between the surface of the heart and the visceral epicardium directly surrounding the three main coronary arteries. Peri-coronary fat thickness (mm) was quantified on axial views of a regular Philips CT workstation. In order not to overestimate the peri-coronary fat due to obliquity, thickness measurements were performed on images in which the axial sections were perpendicular to the surface of the heart. In each of the regions of the main coronary arteries (i.e. right coronary artery,left coronary artery and left circumflex), maximal fat thickness assessed as the largest distance from myocardium to visceral epicardium was determined (Figure 1B). Mean thickness of the peri-coronary fat surrounding the three coronary arteries was used for analyses ('peri-

coronary fat thickness'). The intra- and interobserver coefficient of variations, measured for 60 CT scans, were 12.0% and 13.1%, respectively.[18]

Conventional coronary angiograms were recorded in multiple projections for left and right coronary arteries, and reviewed for significant coronary artery obstructions by cardiologists unaware of the amount of EAT and peri-coronary fat. The severity of coronary atherosclerosis was classified as the number of coronary arteries (right coronary artery, left coronary artery and left circumflex) with a ≥50% luminal diameter stenosis.[19] Subjects were classified as having 0, 1, 2 or 3-vessel disease. Left main artery stenosis was scored as 2-vessel disease.

CAC imaging was performed using multi-slice CT scanning. Non-contrast scans, with 3 mm slice-thickness and an increment of 1.5 mm, were performed during a single breath-hold. The CAC score was obtained using dedicated software for calcium scoring (Heartbeat-CS, Extended Brilliance Workspace, Philips Medical Systems, Best, The Netherlands). A single experienced investigator blinded to clinical data performed the CAC scoring using the Agatston method.[20] All regions with a density >130 Hounsfield Units were identified as potential calcifications. To reduce the influence of noise, the minimum size of calcified lesion was set at 0.5 mm^2. The peak density in Hounsfield Units and the area in mm2 of each selected region were calculated and multiplied by a weighting factor.[20] The scores of individual lesions were added to obtain the Agatston calcium score for the entire coronary tree.
CAC outcome was expressed as ordinal categories, based on cut-offs that have been widely used in the literature and are proposed by Rumberger et al.[21]: ≤10 (minimal or non-significant CAC), 11-100 (mild CAC), 101-400 (moderate CAC), 401-1000 (severe CAC), and >1000 AU (extensive CAC).[21]

Differences in mean EAT volume and peri-coronary fat thickness between patients with 0, 1, and ≥2-vessel disease, and between patients with various CAC scores were determined using ANCOVA (Analysis of Covariance, general linear model procedure) in order to adjust for all variables that were considered to be confounders: age (continuously), and gender. The variables EAT volume and peri-coronary fat thickness were normally distributed. To assess the independent relation of EAT and peri-coronary fat with severity of CAD, we additionally adjusted for BMI (continuously). The modifying effect of BMI on the relationship of EAT volume and peri-coronary fat thickness with severity of coronary atherosclerosis and extent of

CAC was examined by calculating the differences in mean EAT volume and peri-coronary fat thickness for the different subgroups of vessel disease and CAC scores in patients with low and high BMI (cut off point: median BMI 27 kg/m^2).
To reduce bias and increase statistical efficiency, missing values (high-density lipoprotein (HDL)-cholesterol (n = 21), triglycerides (n = 20), fasting glucose (n = 5), creatinine (n = 5), diastolic (n = 12) and systolic (n = 12) blood pressure) in the data were completed by regression imputation.[22,23] Significance was taken at the 5% level (two-sided).

Results

In Table 1 the clinical characteristics of the study population are listed according to tertiles of EAT volume. The distribution of characteristics among tertiles of peri-coronary fat thickness was comparable to the distribution among tertiles of EAT volume (data not shown). Most patients were male (70%), and the mean age of all patients was 61 ± 6 years. Sixty-three (49%) and 29 (23%) patients had a BMI ≥27 and ≥30 kg/m^2, respectively. The prevalence of metabolic syndrome was 43%.

Mean EAT volume was 110 ± 44 cm^3 (range 28-271 cm^3); 110 ± 43 cm^3 for male and 109 ± 48 cm^3 for female patients. Average peri-coronary fat thickness around the coronary arteries was 10.4 ± 2.0 mm. Peri-coronary fat thickness was higher in the right coronary artery region (15.9 ± 3.1 (range 8.9-25.5) mm), compared to the left circumflex region (10.0 ± 2.2 (range 4.3-15.8) mm); paired t-test p-value <0.001, and the left coronary artery region (5.2 ± 1.9 (range 1.7-10.9) mm); paired t-test p-value <0.001.

A total of 109 (85%) patients had a ≥50% stenosis in at least one major coronary artery. EAT volume and peri-coronary fat thickness were not associated with the number of stenotic coronary arteries, adjusted for age and gender (Table 2). Additional adjustment for BMI did not markedly change the results. Among patients with a BMI <27 kg/m^2, those with ≥2 vessel disease (multi-vessel disease) had a higher EAT volume compared to those without CAD (Table 2).

The median CAC score was 181 (37-544). EAT volume and peri-coronary fat thickness were not related to the extent of CAC in the whole study population, adjusted for age and gender (Table 3). Additional adjustment for BMI did not alter the relationship. In patients with a BMI <27 kg/m^2, those with severe or extensive

CAC had a higher EAT volume and peri-coronary fat thickness compared to those with minimal or absent CAC (Table 3).

Table 1. General characteristics of the study population according to tertiles of epicardial adipose tissue volume (n = 128).

Epicardial adipose tissue volume (Range)	Tertile 1 (27.9-84.9 cm^3)	Tertile 2 (86.1-127.1 cm^3)	Tertile 3 (129.3-270.7 cm^3)
	N = 43	N = 43	N = 42
Epicardial adipose tissue volume (cm^3)	64 ± 14	105 ± 12	161 ± 28
Age (years)	59 ± 6	62 ± 5	61 ± 6
Men	28 (65%)	31 (72%)	30 (71%)
Current smokers*	13 (30%)	17 (40%)	12 (29%)
Diabetes mellitus†	5 (12%)	11 (26%)	8 (19%)
Creatinine clearance (ml/min/1.73m^2)‡	81 ± 17	92 ± 21	93 ± 29
Metabolic syndrome (Adult Treatment Panel III)	13 (30%)	20 (47%)	22 (52%)
Body mass index (kg/m^2)	26 ± 3	28 ± 3	30 ± 3
Systolic blood pressure (mmHg)	148 ± 25	155 ± 22	151 ± 19
Diastolic blood pressure (mmHg)	82 ± 14	82 ± 12	78 ± 13
Glucose (mmol/l)	5.4 (5.0-6.2)	6.0 (5.1-7.2)	5.9 (5.4-7.3)
Triglycerides (mmol/l)	1.38 (0.98-2.22)	1.65 (1.05-2.10)	1.40 (1.06-2.14)
Triglycerides (mg/dl)	122 (87-196)	146 (93-186)	123 (94-189)
HDL-cholesterol (mmol/l)	1.37 (1.10-1.56)	1.22 (1.01-1.42)	1.28 (1.02-1.69)
HDL-cholesterol (mg/dl)	53 (42-60)	47 (39-55)	49 (39-65)
Use of Blood pressure-lowering agents	38 (88%)	38 (88%)	39 (93%)
Use of Lipid-lowering agents	32 (74%)	35 (81%)	33 (79%)
Stable angina pectoris	37 (86%)	29 (67%)	34 (81%)
Previous unstable angina pectoris	2 (5%)	3 (7%)	3 (7%)
Previous myocardial infarction	13 (30%)	6 (14%)	10 (24%)

HDL: high-density lipoprotein. All data in n (%), mean ± S.D. or median (interquartile range).
* Still smoking or recently (<1 month before study inclusion) stopped smoking; † Self-reported diabetes mellitus
‡ According to Cockroft-Gault.

Table 2. Epicardial adipose tissue volume and peri-coronary fat thickness in relation to the number of stenotic coronary vessels (≥50% stenosis) in all patients and in subpopulations stratified by total body weight.

Variable	Number of Coronary Arteries Narrowed ≥50%			P-value for trend
	0	1	≥2	
All patients (N = 128)	19 (15%)	54 (42%)	55 (43%)	
Epicardial adipose tissue volume (cm^3)*	104 ± 11	110 ± 6	111 ± 6	0.9
Peri-coronary fat thickness (mm)*	10.1 ± 0.5	10.3 ± 0.3	10.5 ± 0.3	0.7
Patients stratified by body mass index				
Body mass index <27 (N = 65)	10 (15%)	27 (42%)	28 (43%)	
Epicardial adipose tissue volume (cm^3)*	67 ± 12	88 ± 7	100 ± 6	0.04
Peri-coronary fat thickness (mm)*	8.4 ± 0.5	9.2 ± 0.3	9.8 ± 0.3	0.06
Body mass index ≥27 (N = 63)	9 (14%)	27 (43%)	27 (43%)	
Epicardial adipose tissue volume (cm^3)*	155 ± 15	134 ± 8	120 ± 9	0.2
Peri-coronary fat thickness (mm)*	12.5 ± 0.6	11.5 ± 0.3	11.2 ± 0.3	0.2

All data in mean ± se. * Adjusted for age and gender.
Patients were divided according to total body weight based on median body mass index (27 kg/m^2).

Table 3. Epicardial adipose tissue volume and peri-coronary fat thickness in relation to coronary artery calcium (according to Agatston) in all patients and in subpopulations stratified by total body weight.

Variable	Coronary Artery Calcium				P-value for trend
	≤ 10	11-100	101-400	≥ 401	
All patients (N = 128)	21 (16%)	28 (22%)	37 (29%)	42 (33%)	
Epicardial adipose tissue volume (cm^3)*	112 ± 10	111 ± 8	102 ± 7	115 ± 7	0.6
Peri-coronary fat thickness (mm)*	10.4 ± 0.4	10.5 ± 0.4	10.0 ± 0.3	10.7 ± 0.3	0.4
Patients stratified by body mass index					
Body mass index <27 (N = 65)	10 (15%)	15 (23%)	20 (31%)	20 (31%)	
Epicardial adipose tissue volume (cm^3)*	69 ± 10	90 ± 9	82 ± 7	108 ± 7	0.02
Peri-coronary fat thickness (mm)*	8.2 ± 0.5	9.7 ± 0.4	8.8 ± 0.3	10.0 ± 0.3	0.01
Body mass index ≥27 (N = 63)	11 (17%)	13 (21%)	17 (27%)	22 (35%)	
Epicardial adipose tissue volume (cm^3)*	160 ± 14	134 ± 12	129 ± 10	115 ± 10	0.1
Peri-coronary fat thickness (mm)*	12.7 ± 0.5	11.3 ± 0.5	11.4 ± 0.4	11.1 ± 0.4	0.1

All data in mean ± se. * Adjusted for age and gender. Patients were divided according to total body weight based on median body mass index (27 kg/m^2).

Discussion

In this study, it is shown that EAT and peri-coronary fat, quantified using cardiac CT, are not related to the severity of coronary atherosclerosis and extent of CAC in patients suspected of CAD. However, in patients with a low BMI, EAT volume and peri-coronary fat thickness are related to a higher number of stenotic coronary vessels and more severe CAC.

It can by hypothesized that adipose tissue surrounding the heart may contribute to the progression of coronary atherosclerosis due to its proximity to coronary arteries and potential for local secretion of adipocytokines.[4;5] Accordingly, in porcine coronary arteries in vivo, adventitial treatment with inflammatory mediators resulted in the migration of macrophages into the vascular wall and intima thickening.[24;25] In the present study, we indeed found that an accumulation of EAT (volume) and peri-coronary fat (thickness), measured with cardiac CT, was related to an increased severity of CAD in patients with a low BMI.

In the present study 2 different methods were used for the assessment of the severity of CAD: the severity of coronary atherosclerosis (the number of coronary arteries with ≥50% stenosis), which roughly estimates the luminal coronary burden; and the extent of CAC, which also measures early atherosclerotic changes before luminal stenosis occurs. Extent of CAC can be applied as an estimate of the severity of CAD; it is associated with the probability of coronary artery stenosis and related to the risk of developing CAD and the overall coronary plaque burden.[26-28] Moreover, two different estimates of fat accumulation surrounding the coronary arteries, measured with cardiac CT, were used. Namely, a volumetric method of EAT to accurately estimate the total amount of EAT. In addition, a peri-coronary fat thickness measurement to assess the fat thickness directly surrounding the coronary arteries which is conceptually most interesting. EAT and peri-coronary fat are accurately visualised on CT because the fibrous pericardium can be easily visualised due to the high spatial resolution.[29] It should be noted that echocardiography is a simple and easily accessible tool to measure fat around the heart, however, it can not give an adequate window of all cardiac segments and is highly dependent on acoustic windows, which are often inadequate for subtle assessments in obese patients.[29]

Previous studies evaluating the relation between EAT and angiographic CAD provided conflicting results probably due to differences in measurement techniques and study populations.[7;11;12] A positive correlation was shown between EAT thickness and severity of CAD in mainly non-obese patients (mean BMI 24 ± 3 kg/m2) with known CAD7 and in mainly non-obese patients (mean BMI 25 ± 3 kg/m2) undergoing coronary angiography due to chest pain.12 In contrast, another study did not detect an association between EAT thickness and angiographic CAD.11 That study mainly included patients with obesity (BMI ±30 kg/m2). These three studies[7;11;12] used a single echocardiographic thickness measurement to estimate the EAT quantity. The present study is unique in the sense that it adds an accurate volumetric assessment of the total amount of EAT and a thickness measurement of peri-coronary fat, measured with cardiac CT, which were both associated with an increased severity of CAD in patients with a low BMI. Interestingly, it has been shown that pericardial fat (EAT and paracardial fat) volume, measured with CT, was related to angiographic CAD in patients with a BMI <25 kg/m^2.[10] Neither pericardial fat nor BMI, and visceral abdominal fat were independently correlated with CAD in patients with a BMI ≥25 kg/m2.

In the present study, the lack of an association of EAT volume and peri-coronary fat thickness with severity of CAD in the whole study population, may be the result of effect modification by body weight. In line with previous findings[10], our results indicate that there might be a differential effect of fat surrounding coronary arteries (regional body fat) on CAD in patients with low and high BMI. This may be due to several reasons. Although adipose tissue around the heart is strategically located close to the coronary arteries, it is a relatively small visceral fat depot compared to abdominal fat. Patients with a high BMI are often insulin resistant leading to higher systemic plasma levels of inflammatory cytokines thereby accelerating atherogenesis. In that case, the local production of adipocytokines by epicardial adipocytes adds little to the inflammatory process in the vessel wall. This reasoning is supported by the absence of an association between EAT thickness and angiographic CAD in a study where 40% of the patients had a BMI >30 kg/m^2. [12] Moreover, patients with a high BMI are generally obese and therefore also have an increased amount of EAT or peri-coronary fat. In case of the absence of insulin resistance, adipocytes do not produce large quantities of inflammatory cytokines and

therefore this adipose tissue does not lead to large metabolic abnormalities (e.g. hypertension, hyperglycemia, and low HDL-cholesterol and adds little to the atherosclerotic process. Further studies are needed to clarify this.

We acknowledge potential limitations of the present study. Firstly, due to the cross-sectional study design only assumptions about possible etiological relationships could be made. Secondly, although the definitions for severity of CAD (the number of coronary arteries with a ≥50% luminal diameter stenosis and extent of CAC) are often used in the literature[19;20] they do not account for the presence of plaque instability which may lead to acute coronary artery stenosis. Furthermore, waist circumference was not measured; therefore we could only stratify for total body weight according to BMI. However, fat surrounding coronary arteries was related to waist circumference and BMI [6;7] which are both indicators of a high cardiovascular risk.[8] Therefore, stratifying for waist circumference will not change the direction of our results, but it might change the magnitude. Lastly, the functional characteristics of fat surrounding the coronary arteries may be more important in the pathophysiology of CAD than the amount.

In conclusion, EAT and peri-coronary fat are related to more severe coronary atherosclerosis and CAC in patients suspected of CAD with a BMI <27 kg/m2.

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Chapter 10

General discussion

General discussion

Non-invasive cardiac imaging in patients with peripheral arterial disease

Coronary artery disease is one of the most important causes of death [1;2]. Patients with peripheral arterial disease (PAD) are at increased risk of suffering a coronary event [3-5]. In the first part of this thesis we describe the design of a study on how subjecting patients with PAD, but without cardiac symptoms, to an imaging algorithm of CT angiography and dobutamine stress MRI (DSMR), followed by subsequent treatment may reduce the risk of suffering a coronary event. The finding that one fifth of the patients had evidence of severe coronary atherosclerosis that forms a class I indication for revascularization in combination with the relatively little amount of positive DSMR's made the principle investigators of the trial stop the inclusion of patients for ethical reasons.

The latest guideline for coronary artery bypass surgery states that having a coronary left main stenosis (or its equivalent), even in the absence of cardiac symptoms, is a Class I indication for surgical revascularization[6]. This statement is based upon three randomized trials[7-9] and several smaller studies[10-12]. A meta-analysis of these studies showed a survival benefit for CABG of 19.3 months in patients with left main stenotic disease[13]. In the CASS registry (Coronary Artery Surgery Study), after 15 years, survival of patients who underwent CABG was 44%, whereas survival in patients who did not undergo CABG was only 31%[8;14] and in asymptomatic left main disease 5-year-survival was 88% in the CABG treated group versus 58% in the medically treated group[15]. Important limitations of these trials have to be acknowledged. First, the trials date back to a time when both aspirin and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) were not routinely used or available. The same applies to angiotensin converting enzyme inhibitors (ACE-inhibitors). Both these pharmacologic agents have shown to reduce vascular risk in a large variety of symptomatic and asymptomatic patients. Participants were included in the 1970's and 80's when the predominant medical therapy consisted of beta-blockers and nitrates. Patients included in the CASS registry had a history of myocardial infarction or a positive stress test and therefore had evidence of silent ischemia. Therefore it is presently not clear from the literature whether truly asymptomatic patients with a left main stenosis would benefit from revascularization. On the other hand, today different and less invasive approaches to

bypass surgery are used with lower peri-procedural morbidity and mortality as compared to bypass surgery in the 1980's. For example in the time of the above mentioned studies the use of the internal mammary artery was rare. Most participants in these studies were men (96.8%) and mean age was more than 11 years younger than in the GROUND study. The Asymptomatic Cardiac Ischemia Pilot (ACIP) study demonstrated that revascularization had better outcome than medical therapy in asymptomatic patients with CAD[16]. However, even though the study population consisted of asymptomatic patients, all participants had evidence of cardiac ischemia on stress testing[17], implying that the participants are further down the ischemic cascade and thus are at higher risk and may therefore benefit more from revascularization than the GROUND study participants [6]. We conclude that it is unclear whether the results of these studies, on which the guidelines are based, are generalizable to the GROUND study population of PAD patients.

DSMR has proven to be a functional test of superior quality in detecting inducible cardiac ischemia in both symptomatic and asymptomatic patients [18-20]. In the GROUND study only 2 patients had signs of inducible ischemia on DSMR, even though the burden of coronary artery disease was found to be very high (chapter 4). A possible explanation may be that the prevalent stenoses, as seen on CT angiography, evolved gradually. Participants of the GROUND study are on average 62 years old. This could have allowed for enough time for coronary collaterals to form. Unfortunately, current CT scanners do not allow imaging coronary collaterals in a reliable way yet [21]. However, both CT scanners and post-processing software are developing rapidly.

The question whether the imaging algorithm of the GROUND study will lead to less coronary events will not be answered in GROUND due to lack of power. Studies of this type are scarce and today there is not enough evidence that subjecting this patient group to an imaging algorithm of any type may help to reduce risk of cardiac morbidity or mortality. Therefore we can not call upon screening these patients with the algorithm presented in this thesis. However, further study into potential screening with modern non-invasive imaging modalities is necessary given the very high risk of coronary events in this particular patient group. We showed that many patients suffer form different atherosclerotic plaque types in their coronary arteries. Follow up data will help us further in exploring the prognostic

consequences of these findings. It is clear from risk factor management guidelines that patients with PAD should receive optimal risk factor treatment, i.e. aspirin, a statin and antihypertensive medication, if indicated and counseling with regard to physical exercise, healthy diet and cessation of smoking [22]. However, studies show that patients with PAD are undertreated with regard to this risk factor modification [23;24].

The management of patients with stable CAD remains debatable. In 2007 results from the Clinical Outcomes and Aggressive Drug Evaluation (COURAGE) study were published, which revealed that in patients with stable CAD, percutaneous coronary intervention (PCI) did not reduce the risk of death, myocardial infarction, or other major cardiovascular events when added to optimal medical therapy [25]. Unfortunately, patients with a left main stenosis were not included in the study. Since we demonstrate such a high prevalence of left main stenoses in the GROUND population, it is unclear whether results from the COURAGE study can be used for the management of patients with PAD.

In conclusion, many patients with cardiac asymptomatic PAD, who are known to be at very high risk of suffering a coronary event, have a significant stenosis in the left main coronary artery. Although this is a Class I revascularization indication according to the current guidelines, in the literature there is growing evidence that patients with chronic stable coronary artery disease may not benefit from intervention when compared to current medical prevention strategies. It is unclear whether this is also applies to left main disease. Therefore, it may be defensible to initiate a large randomized clinical trial in which patients with cardiac asymptomatic PAD are screened with coronary CT angiography after randomization to either a group who will undergo revascularization in case a left main stenosis is found or to conservative treatment in order to answer two questions: Firstly, whether screening is beneficial to cardiac asymptomatic patients with PAD. Secondly, whether patients with chronic stable left main disease should undergo revascularization. Until such a trial has been performed we call upon intensive medical risk factor modification in this patient group.

Epicardial adipose tissue and coronary atherosclerosis

In the second part of this thesis we explored the use of cardiac MDCT in the assessment of epicardial adipose tissue (EAT) and peri-coronary EAT and its possible role in the development of atherosclerosis. Obesity is becoming an important health problem in the western society [26]. Adipose tissue was long regarded as passive fuel storage. However, recent studies have revealed that adipocytes release numerous biologically active molecules into the circulation and play a role in the development of atherosclerosis in obese persons [27]. Studies have shown that EAT is a source of pro-inflammatory cytokines, such as tumor necrosis factor α, interleukin 6 and monocyte chemotactic protein 1 (MCP-1), surrounding the coronary arteries and that this depot of visceral fat has a higher rate of secreting these cytokines than subcutaneous fat [28]. Animal studies have shown how adventitial application pro-inflammatory cytokines results in intimal thickening through the migration of macrophages from 'outside to inside' into the vessel wall [29;30]. Furthermore, coronary segments which lack peri-coronary EAT due to myocardial bridging, show no atherosclerosis [31;32].

Most studies visualized EAT with the use of echocardiography [33;34]. However, CT has a much higher resolution[35]. In this thesis we showed that the total amount of EAT around the heart can be quantified on CT angiography scans in a highly reproducible way. Measurements are relatively time consuming, but may be done in the future with the use of dedicated software programs. Unfortunately, CT scans of this type are associated with the use of potentially nephrotoxic contrast agents and relatively high radiation dose. However, peri-coronary EAT can be quantified on low radiation dose CT calcium scoring scans in approximately 3 minutes. These measurements are done on a regular CT working station. However, this method of measuring peri-coronary EAT has been shown to be less reproducible than measuring total EAT. From a pathophysiologic perspective peri-coronary EAT is more interesting, since it is located in the direct proximity of the coronary arteries.

Before we started our work, several studies on the relation between pericardial fat (all the fat around the heart) and coronary artery disease were available, but results were conflicting [36;37]. In a cross-sectional study among healthy post-menopausal women, we found relations with cardiovascular risk factors. In addition, we found a relation between peri-coronary EAT and coronary calcification. In a group of patients with manifest coronary artery disease, we found these

relations only in participants with a low body mass index. These findings support the theory that EAT is involved in the process of atherosclerosis. This was recently further supported by a study in the Framingham Heart Study Offspring cohort, in which both pericardial and abdominal visceral adipose tissue were associated with the prevalence of cardiovascular disease, independent of traditional measures of obesity, such as body mass index and waist circumference [38].

The metabolic syndrome may be related to coronary atherosclerosis because of the fact that obese persons are prone to be insulin resistant [39]. Insulin resistance is associated with high levels of circulating pro-inflammatory adipocytokines, which are secreted from visceral adipose tissue and were recently found to be associated with coronary atherosclerosis [40]. In post-menopausal women we found a relation between the metabolic syndrome and peri-coronary EAT. Furthermore, a graded relation was found between the number of metabolic syndrome components and peri-coronary EAT. The strongest metabolic syndrome component in this relation was waist circumference. Yet, in patients with lower than median waist circumference the relation of peri-coronary EAT with the metabolic syndrome persisted. This finding may suggest that fat around the coronary arteries is more than just a marker of abdominal visceral adiposity and might point towards an alternative, complementary mechanism to explain the relation between metabolic syndrome and increased cardiovascular risk. The metabolic syndrome may comprise increased risk of CAD influenced by the presence of peri-coronary EAT [41], because EAT is a rich source of pro-inflammatory adipocytokines [28].

Future studies are needed to better understand the exact etiologic role of peri-coronary EAT and also follow up studies are needed to determine if we are dealing with a new risk factor for the development of coronary artery disease. In such studies adjustment has to be done for other regions of visceral fat, such as abdominal fat, in order to find the prognostic value of having fat around the coronary arteries. In the future, EAT may prove to be a better predicting risk factor for coronary artery disease than other fat measures, such as body mass index and waist circumference. It may have an incremental value to traditional cardiovascular risk factors and could eventually become a potential target for pharmacologic therapy.

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Chapter 11

Summary

Samenvatting

Summary

The number of patients with coronary artery disease is very high in our society. In the past decade non-invasive cardiac imaging modalities such as multi-detector computed tomography (MDCT) have evolved rapidly. Visualization of coronary arteries and its direct surrounding is now possible with relatively little hazard to the patient. In this thesis, we focused on non-invasive cardiac imaging in cardiac asymptomatic patients with peripheral arterial disease (PAD) and its role in assessing epicardial adipose tissue (EAT), a potential risk factor for the development of coronary atherosclerosis.

Chapter 1 is an introduction to the work presented in this thesis. We describe that patients with PAD who do not have cardiac symptoms are at high risk of coronary artery disease. We also describe how non-invasive cardiac imaging such as MDCT and dobutamine stress MRI (DSMR) have opened the way to screening these cardiac asymptomatic high risk patients. In the second half we focus on the role of EAT as a potential risk factor for the development of coronary atherosclerosis. EAT has the same origin as abdominal visceral fat and it is a source of bioactive molecules in the direct surrounding of the coronary artery vessel wall. Studies show how EAT may play a role in the development of coronary atherosclerosis. MDCT has the potential to visualize this peri-coronary EAT.

In **chapter 2** we present the rationale and design of the GROUND study. The GROUND study was a prospective, multi-center, randomized clinical trial, performed in 4 large hospitals in the Netherlands. Patients with PAD, but without symptomatic cardiac disease were randomized to undergo either CT calcium scoring only or to undergo a non-invasive cardiac imaging algorithm consisting of CT calcium scoring, coronary CT angiography and DSMR testing. Patients with a left main (or equivalent) coronary artery stenosis of >50% on CT did not undergo DSMR testing, but were referred to a cardiologist. Also patients with a positive DSMR were referred to a cardiologist. These referred patients were candidates for conventional coronary artery angiography and cardiac interventions, such as coronary artery bypass grafting (CABG) or percutaneous cardiac interventions (PCI). All participants of the study received proper risk factor modification and life style advice according to the current guidelines. Also, all participants entered a 5 year follow up period for the occurrence of cardiovascular events. The aim of the study was to evaluate

whether a modern imaging algorithm consisting of CT and DSMR can help to reduce cardiac morbidity and mortality in a cardiac asymptomatic high risk population.

In **chapter 3** we present baseline results of 231 participants of the GROUND study. We demonstrate that one fifth of cardiac asymptomatic patients with PAD have a significant stenosis in the left main coronary artery or its equivalent on cardiac CT angiography. A stenosis in this area is considered a class I indication for revascularization. Only 2 out of 76 participants who underwent DSMR had a positive test result. Finding this large amount of left main stenoses was enough reason for the principal investigators of the study to stop the inclusion of patients. This finding may have major implications for the management of patients with cardiac asymptomatic PAD. The question, however, of whether the imaging algorithm of the GROUND study, MDCT in combination with DSMR, can help to reduce morbidity and mortality in this patient group, will likely not be answered due to lack of power.

In **chapter 4** we demonstrate the prevalence and localization of non-calcified, mixed and calcified plaque in the coronary arteries of the GROUND study population. The most important findings are that more than 90% of the participants show coronary atherosclerosis and half of the patients has a significant stenosis in their coronary arteries. Also half of the patients showed soft plaque in their coronary arteries. The most prevalent localization of coronary atherosclerosis is the left anterior descending coronary artery and the proximal rather than the distal coronary arteries.

In **chapter 5** with the use of CT calcium scoring, we show a considerable amount of calcium in the coronary arteries of the GROUND study population. We found a 6 times increase in the risk of having a class I revascularization indication for diabetics and a more than 12 times increase of this risk in patients with an Agatston calcium score of more than 400. Diabetic status and the use of calcium scoring may help in selecting PAD patients at highest risk of suffering a cardiac event.

In **chapter 6** we show that with the use of cardiac CT the amount of EAT around the heart can be quantified in a reliable way. Also the EAT around the coronary arteries and on the right ventricular free wall can be measured, albeit in a less reproducible way. In this study, performed in patients suspected of coronary artery stenosis, epicardial adipose tissue was related to obesity.

In **chapter 7** we demonstrated that in a population of healthy post-menopausal women peri-coronary EAT was related to multiple known cardiovascular risk factors, with waist circumference being the strongest factor. Peri-coronary EAT was also related to calcium scoring as a sign of coronary atherosclerosis. These findings support the theory that EAT may play a role in the development of coronary atherosclerosis.

The metabolic syndrome is a cluster of unfavorable cardiovascular risk factors associated with cardiovascular events. In **chapter 8** we found that in post-menopausal women peri-coronary EAT is related to the metabolic syndrome. Furthermore, there was a graded relation between the number of metabolic syndrome factors and peri-coronary EAT.

In **chapter 9** we were not able to show a relation between severity of coronary atherosclerosis and EAT in patients suspected of coronary artery disease. However, in patients with a low body mass index, high amount of total EAT around the heart or peri-coronary EAT were related to severity of coronary atherosclerosis and calcium score, indicating that fat surrounding the coronary arteries may be involved in the process of atherosclerosis.

Finally, in **chapter 10** the results of the studies presented in this thesis are discussed.

Samenvatting

Hart- en vaatziekte behoort tot de belangrijkste doodsoorzaken in westerse landen. In de afgelopen jaren heeft niet-invasieve cardiale beeldvorming als multi-detector CT (MDCT) zich in hoog tempo ontwikkeld. Het in beeld brengen van de kransslagaders en de directe omgeving hiervan behoort nu tot de mogelijkheden. In dit proefschrift richten we ons op niet-invasieve cardiale beeldvorming bij cardiaal asymptomatische patiënten met perifeer vaatlijden en op het in beeld brengen van epicardiaal vet als een potentiële risicofactor voor het ontwikkelen van coronair lijden (slagaderverkalking).

Hoofdstuk 1 is een inleiding tot de hoofdstukken van dit proefschrift. Wij beschrijven dat patiënten met perifeer vaatlijden een zeer hoog risico hebben op het ontwikkelen van coronair lijden. Tevens wordt beschreven hoe we met niet-invasieve cardiale beeldvorming als MDCT en dobutamine stress MRI (DSMR) in deze hoog risico groep de aanwezigheid van atherosclerotische afwijkingen in de kransslagaders (coronairen) en mogelijk zuurstoftekort in de hartspier kunnen bestuderen. In de tweede helft richten we ons tot de rol van epicardiaal vet als een mogelijke risicofactor voor het ontwikkelen van coronaire slagaderverkalking. Vet rond de coronairen heeft dezelfde herkomst als abdominaal visceraal vet en het is een rijke bron van diverse bioactieve moleculen in de directe omgeving van de wand van de kransslagaders. Uit studies blijkt hoe deze moleculen een mogelijke rol spelen bij de ontwikkeling van slagaderverkalking. Met MDCT kan dit epicardiale vet en slagaderverkalking in beeld worden gebracht.

In **hoofdstuk 2** presenteren wij de GROUND studie. De GROUND studie was een prospectieve, multi-center, gerandomiseerde klinische trial, uitgevoerd in 4 grote ziekenhuizen in Nederland naar het effect van uitgebreide evaluatie van het hart ten opzichte van gebruikelijke zorg op het krijgen van een hartvaatziekte. Patiënten met perifeer vaatlijden zonder cardiale voorgeschiedenis werden gerandomiseerd naar ofwel de ‘controle groep’, ofwel de ‘behandelgroep’. In de ‘controlegroep’ werd slechts een kalkscore scan gemaakt en patiënten in de behandelgroep werden blootgesteld aan een algoritme bestaande uit een kalkscore scan, CT angiografie en een DSMR van het hart. Patiënten met een vernauwing ter hoogte van de hoofdstam op CT, ondergingen geen DSMR, maar werden verwezen naar de cardioloog. Ook patiënten met een positieve DSMR werden verwezen naar de cardioloog. Deze

patiënten kwamen in aanmerking voor coronair angiografie en mogelijke cardiale interventies zoals een bypass operatie of een dotterbehandeling. Alle deelnemers aan de studie kregen adequate behandeling van risicofactoren volgens de meest recente richtlijnen. Alle deelnemers worden gedurende 5 jaar gevolgd door middel van halfjaarlijkse vragenlijsten. Het doel van dit onderzoek was om er achter te komen of het onderwerpen aan een algoritme van niet-invasieve beeldvorming het risico op morbiditeit en mortaliteit kan reduceren in deze groep van cardiaal asymptomatische patiënten met hoog risico.

In **hoofdstuk 3** presenteren wij eerste resultaten van de 231 deelnemers aan de GROUND studie. We laten zien dat een vijfde van deze patiënten met perifeer vaatlijden een significante stenose heeft ter hoogte van de hoofdstam (of equivalent). Een stenose in dit gebied wordt beschouwd als een klasse 1 indicatie voor revascularisatie. Slechts 2 van de 76 deelnemers die een DSMR ondergingen hadden een positief test resultaat. De hoge prevalentie aan stenoses ter hoogte van de hoofdstam (of equivalent) was aanleiding voor de hoofdonderzoekers van de studie om de inclusie te staken. Deze bevinding kan verregaande gevolgen hebben voor de behandeling van deze cardiaal asymptomatische groep patiënten. Echter, de originele onderzoeksvraag of deze combinatie van beeldvorming het risico op morbiditeit en mortaliteit kan doen afnemen, zal waarschijnlijk niet worden beantwoord.

In **hoofdstuk 4** laten wij de prevalentie en lokalisatie van niet-gecalcificeerde, gemengde en gecalcificeerde plaque in de coronair vaten van de GROUND populatie zien. De belangrijkste bevindingen zijn dat meer dan 90% van de patiënten tekenen van coronair lijden heeft en de helft heeft een significante stenose ergens in het coronaire stelsel. Tevens heeft de helft van de patiënten een niet-gecalcificeerde plaque in de coronairen. Atherosclerose kwam het meest voor in de linker kransslagader.

In **hoofdstuk 5** tonen wij dat mensen met perifeer vaatlijden zonder cardiale klachten op een CT kalkscore scan veel kalk in de coronairen hebben. We vonden dat patiënten met diabetes mellitus een 6 keer verhoogd risico hebben op de aanwezigheid van een stenose van de hoofdstam en voor patiënten met een kalkscore van meer dan 400 was dit risico zelfs 12 keer verhoogd. Het hebben van

diabetes mellitus en het gebruik van CT kalkscore kan helpen bij het selecteren van patiënten met perifeer vaatlijden met het hoogste risico op coronair lijden.

In **hoofdstuk 6** laten wij zien hoe met cardiale CT op reproduceerbare wijze de hoeveelheid EAT rond het hart kan worden gekwantificeerd. Ook de hoeveelheid EAT in de directe omgeving van de coronairen kan worden gemeten. Hoewel deze laatste meting pathofysiologisch interessanter is, is hij wel minder reproduceerbaar. In deze studie in patiënten die verwezen waren voor coronair angiografie in verband met verdenking op stenosering van de coronairen bleek de hoeveelheid EAT gerelateerd aan overgewicht.

In **hoofdstuk 7** houdt in een populatie van gezonde post-menopauzale vrouwen, de hoeveelheid EAT rond de coronairen verband met ongunstige niveaus van cardiovasculaire risicofactoren. Taille omtrek blijkt de sterkste gerelateerde factor. Tevens bleek peri-coronair EAT gerelateerd aan calcificatie van de vaatwand. Deze bevindingen ondersteunen de theorie dat EAT een mogelijke rol heeft in de ontwikkeling van coronaire atherosclerose.

Het metabool syndroom is een clustering van risicofactoren gerelateerd aan overgewicht en insulineresistentie en is geassocieerd met een sterk verhoogd voorkomen van coronair lijden. In **hoofdstuk 8** vonden wij dat in gezonde post-menopauzale vrouwen peri-coronair EAT gerelateerd is aan het metabool syndroom. Bovendien was er een gegradeerde relatie tussen het aantal factoren waaruit het metabool syndroom bestaat, en de hoeveelheid peri-coronair EAT.

In **hoofdstuk 9** kon in patiënten die verwezen waren voor coronair angiografie geen relatie worden aangetoond tussen de ernst van coronair lijden en EAT. Echter, in patiënten met een lage Quetelet-index bleek wel een relatie tussen coronair lijden en EAT. Dit kan wijzen op een rol van EAT in de pathofysiologie van atherosclerose.

In de algemene discussie in **hoofdstuk 10** bespreken we de bevindingen van dit proefschrift.

Dankwoord

Dankwoord

Iedereen die een bijdrage heeft geleverd aan de totstandkoming van dit proefschrift wil ik hartelijk danken. Zonder iemand tekort te willen doen, noem ik in het bijzonder:

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Curriculum Vitae / List of Publications

Curriculum Vitae

The author was born on March 3rd 1976 in Sliedrecht, the Netherlands. In 1993 he graduated from high school at 'Keene High School', Keene, New Hampshire, U.S.A. Secondary school was completed in 1995 with a gymnasium diploma at the Dr. W.A. Visser 't Hooft Lyceum in Leiden, the Netherlands. He studied medicine at the University of Antwerp, Belgium. During his study he spent one year at the Rheinische Friedrich-Wilhelms-Universität Bonn, Germany, where he came into contact with cardiology. A scientific training followed at the department of experimental cardiology, Erasmus University Medical School in Rotterdam under the supervision of prof.dr. D.J.G.M. Duncker. The topic of research was 'effects of exercise training and eNOS overexpression on left ventricle function after acute myocardial infarction'. In June 2003 he graduated cum laude ('met onderscheiding') in Antwerp. In September of that same year he started as a PhD student at the departments of Radiology and Cardiology of the University Medical Center Utrecht under the supervision of prof.dr. M. Prokop, prof.dr. W.P.Th.M. Mali, prof.dr. P.A.F.M. Doevendans, dr. M.L. Bots and dr. B.J. Rensing. In August 2006 he obtained his Master of Science degree in clinical epidemiology at the Netherlands Institute for Health Sciences (NIHES) in Rotterdam under the supervision of prof.dr. A. Hofman. That same year he started two years of internal medicine training at the Meander Medical Center, Amersfoort under the supervision of dr. C.A. Gaillard. In December 2008 he started his cardiology training at the University Medical Center Utrecht under the supervision of dr. J.H. Kirkels and in December 2010 he will continue his cardiology training at the Antonius Hospital Nieuwegein under the supervision of dr. W. Jaarsma.

List of publications

Meijboom WB, Meijs MF, Schuijf JD, Cramer MJ, Mollet NR, van Mieghem CA, Nieman K, van Werkhoven JM, Pundziute G, Weustink AC, **de Vos AM**, Pugliese F, Rensing B, Jukema JW, Bax JJ, Prokop M, Doevendans PA, Hunink MG, Krestin GP, de Feyter PJ. Diagnostic accuracy of 64-slice computed tomography coronary angiography: a prospective, multicenter, multivendor study. *J Am Coll Cardiol*. 2008;52:2135-2144.

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List of abbreviations

ABI	ankle brachial index
ANOVA	analysis of variance
ATPIII	third adult treatment panel
BMI	body mass index
CABG	coronary artery bypass grafting
CAC	coronary artery calcification
CAD	coronary artery disease
CAG	coronary angiogram
CHD	coronary heart disease
CRF	case report form
cCTA	cardiac computed tomography angiography
CI	confidence interval
CT	computed tomography
CV	coefficient of variation
CVD	cardiovascular disease
DSE	dobutamine stress echocardiography
DSMB	data safety and monitoring board
DSMR	dobutamine stress magnetic resonance imaging
EAT	epicardial adipose tissue
ECG	electrocardiogram
FFA	free fatty acid
HDL	high density lipoproteins
IC	intermittent claudication
IWMA	inducible wall motion abnormality
LAD	left anterior descending coronary artery
LDL	low density lipoproteins
LM	left main coronary artery
LCX	circumflex coronary artery
MDCT	multi-detector computed tomography
MRI	magnetic resonance imaging
MPR	multiplanar reformation
NCEP	national cholesterol education program
NPV	negative predictive value
OR	odds ratio
PAI-1	plasminogen activator inhibitor-1
PAD	peripheral arterial disease
PCI	percutaneous coronary intervention
PPV	positive predictive value
PTCA	percutaneous transluminal coronary angioplasty
RCA	right coronary artery
ROC	receiver operating characteristic
RV	right ventricle
ROI	region of interest
RWMA	rest wall motion abnormality
SD	standard deviation
TNF-α	tumor necrosis factor alpha
WMA	wall motion abnormality