Cardiovascular Risk Assessment in Diabetes

Roxana Djaberi

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For my parents
To Philip and little Mira

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CHAPTER 1

General Introduction

INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycemia. It affects approximately 3% of the population worldwide (1). The criteria for the diagnosis of diabetes have been proposed by the American Diabetes Association (2) and comprise the presence of one of the following conditions: 1. symptoms of diabetes with a concurrent casual plasma glucose of ≥11.1 mmol/l, 2. a fasting plasma glucose concentration of ≥7.0 mmol/l or 3. a two hour glucose post-load level of ≥11.1 during an oral glucose tolerance test.

Several pathogenic processes are involved in the development of diabetes (3,4). The vast majority of patients with diabetes fall into two broad etiopathogenic categories: type 1- and type 2 diabetes. In type 1 diabetes hyperglycemia is caused by an absolute insulin deficiency, and can be identified by serological evidence of a pathologic autoimmune process in the pancreatic islets and by genetic markers (4). The far more prevalent type 2 diabetes, which accounts for 90-95% of cases, encompasses individuals who have insulin resistance and often have relative (rather than absolute) insulin deficiency. Type 2 diabetes is frequently associated with overweight and other components of metabolic syndrome (hypertension and dyslipidemia).

In diabetes, cardiovascular disease constitutes a major cause of morbidity and mortality (5). Long term complications include microvascular impairment in organs (e.g. eyes, heart, kidneys and nerves), as well as macrovascular injuries of the cerebral, coronary and peripheral arterial vasculature.

PATHOPHYSIOLOGY OF CORONARY ARTERY DISEASE IN DIABETES

Previous studies on diabetes and related heart disease have mainly focused on type 2 diabetes (6,7). The metabolic abnormalities that characterize type 2 diabetes, particularly hyperglycemia, free fatty acids, and insulin resistance, provoke various molecular mechanisms: increased oxidative stress, disturbances of intracellular signal transduction (such as activation of protein kinase C), and activation of receptor for advanced glycation endproducts (RAGE) (8,9). Consequently, there is decreased availability of nitric oxide, increased production of endothelin, activation of transcription factors such as nuclear factor-κB and activation protein-1, and increased production of pro-thrombotic factors such as tissue factor and plasminogen activator inhibitor-1 (8,9). In turn, these abnormalities contribute to the cellular events that result in endothelial dysfunction, atherosclerosis and a pro-thrombotic state. In addition, the increased prevalence of coronary artery disease (CAD) in type 2 diabetes is associated with a constellation of risk factors. Patients with type 2 diabetes are frequently obese, and often suffer from hypertension and exhibit dyslipidemia.

The pathophysiology of atherosclerosis in type 1 diabetes has not been fully elucidated (10). However, current thinking supports a model similar to type 2 diabetes in which hyperglycemia, glycation, and oxidation products cause endothelial dysfunction, pro-inflammatory, and pro-thrombotic changes. Also, endothelial and smooth muscle dysfunction can be a precursor for hypertension, which is in turn a major risk factor for CAD. Lipoprotein disturbances are less apparent in type 1 diabetes.

NATURE OF CAD IN DIABETES

CAD in diabetic patients is suggested to be distinct from the non-diabetic patients (11). In type 2 diabetes, evaluation of atheroscleotic plaques in post-mortem studies as well as patients undergoing coronary atherectomy suggest more diffuuse CAD, more vulnerable eccentric plaques, with greater inflammatory cell infiltration (12). However, prior studies often have selection biases, as they are event or procedure driven. Even less information is available concerning CAD in type 1 diabetes (10). A detailed investigation of the nature of CAD, in a representative general population of type 1- and type 2 diabtetes is therefore warranted.

RISK OF CAD IN DIABETES

The level of incremental risk for CAD disease in diabetes has been a topic of discussion in the last decades (13-15). Based on data collected in 1982-1990, Haffner et al. suggested the risk of myocardial infarction to be similar in diabetic patients without previous myocardial infarction as compared to non-diabetic patients with prior myocardial infarction (13). That initial study formed the basis for implementation of aggressive medical treatment of *all* diabetic patients. Accordingly, the 2007 ADA/AHA guidelines recommended treatment with statins and blood pressure lowering medication (16). Also, the use of aspirin in form of primary prevention was suggested in diabetic patients >40 years of age. However, replications of the findings by Haffner et al. have been inconsistent, and recent data suggest a more moderate risk of CAD in diabetes (14-15). It should also be pointed out that major advances have developed in glucose regulation and treatment approaches to diabetes, likely modifying risk of complications. A more recent meta-analysis comparing CAD risk in individuals with diabetes with those with previous myocardial infarction but without diabetes reported lower overall relative odds of CAD events in the individuals with diabetes (14). Of the 13 studies included, 11 reported significantly lower odds of incident CAD among individuals with diabetes (14). Moreover, a recent prospective population-based study in Spain, found a hazard ratio of 0.33 for myocardial infarction in individuals with type 2 diabetes compared with nondiabetic individuals with a prior myocardial infarction (15). Therefore, it seems

diabetes is not the equivalent of previous myocardial infarction with respect to future risk of CAD.
Nevertheless, the incremental risk of CAD in diabetes is high and confers worse prognosis. As compared to the general population, the risk of CAD events is two to four fold in type 2 diabetes. Simillar risk has been reported men with type 1 diabetes (hazard ratio 3.0) (17). Whereas a relative higher risk of CAD events has been observed in women with type 1 diabetes (hazard ratio 7.6). Considering the wide ranging inter-individual variability the question remains how to identify the *high-risk* diabetic patient.

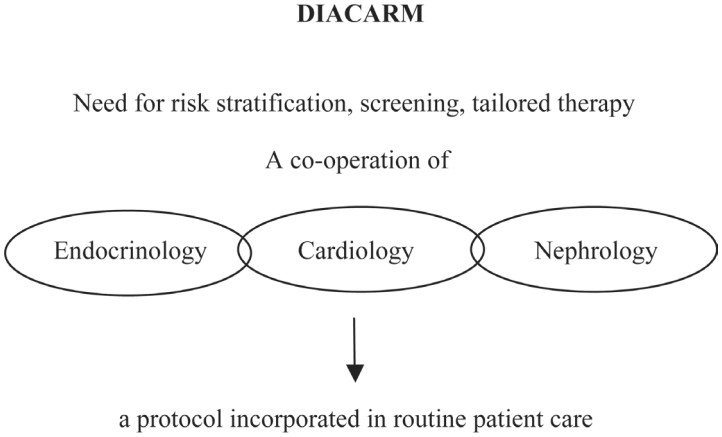
RISK STRATIFICATION FOR CAD IN DIABETES

In diabetes, a wide ranged routine screening strategy of all patients by non-invasive cardiac imaging does not seem cost-effective, and was not shown to influence treatment or outcome (18). A pivotal role could be expected for hyperglycemia in development of vascular damage. Nevertheless, epidemiological evidence does not directly link the level of glycemic control (hemoglobin A 1C) or the duration of hyperglycemia with CAD events. Also, an expert based suggestion by the ADA/AHA in 2000 to consider the presence of ≥ two additional cardiovascular risk factors as a threshold for increased risk of CAD has not been confirmed. This selection strategy was shown to be ineffective in the DIAD study (6), wherein 41% of asymptomatic diabetic patients with abnormal myocardial perfusion did not have ≥ two risk factors.
The clinical utility of risk prediction models may also be limited. The Framingham score has been shown to underestimate event rates in diabetes as compared to the general population (19). Prospective evaluation of the diabetes specific UKPDS suggested less underestimation, but showed a poor relation between actual and predicted CAD events on an individual basis. The SCORE and DECODE models incorporate diabetes in a categorical fashion and do not discriminate risk level in presence of diabetes.
It is assumable that direct estimation of atherosclerosis by means of surrogate markers may provide more accurate risk stratification for the prevalence and incidence of CAD. Indeed, an increased carotid intima media thickness (CIMT) and increased vascular stiffness as assessed by pulse wave velocity (PWV), have been observed in diabetic patients with established CAD (20,21). In a limited number of studies an increased CIMT has also been related with a higher incident rate of CAD events (22,23). However the relation of surrogate markers with silent CAD has not been evaluated in an overall asymptomatic population of diabetic patients. Furthermore, the incremental value of these markers above age and modified risk models, for the prediction of prevalent CAD and CAD events has not been evaluated.
Novel serum biomarkers may convey the additional advantage of reflecting on an individual's predisposition to develop CAD in an early stage. Thereby, patients at risk

for CAD, could be distinguished, prior to evident atherosclerosis and treated more aggressively. In addition, application of a biomarker may provide a more general estimation of atherosclerotic risk, opposing diagnostic techniques which are often restricted to a certain aspect of vascular disease or a specific organ. On the other hand, clinical utility of biomarkers is often limited by their low specificity. Research to attain a single biomarker with sufficient sensitivity as well as specificity for early recognition of atherosclerosis in various sub-populations continues.
Considering the growing number of patients with diabetes, an optimal risk stratification strategy for the selection of the *high-risk* diabetic patient should convey a number of characteristics to enable wide range implementation: sufficient sensitivity and specificity, low costs and limited side-effects (e.g. radiation) as well as a non-invasive nature.

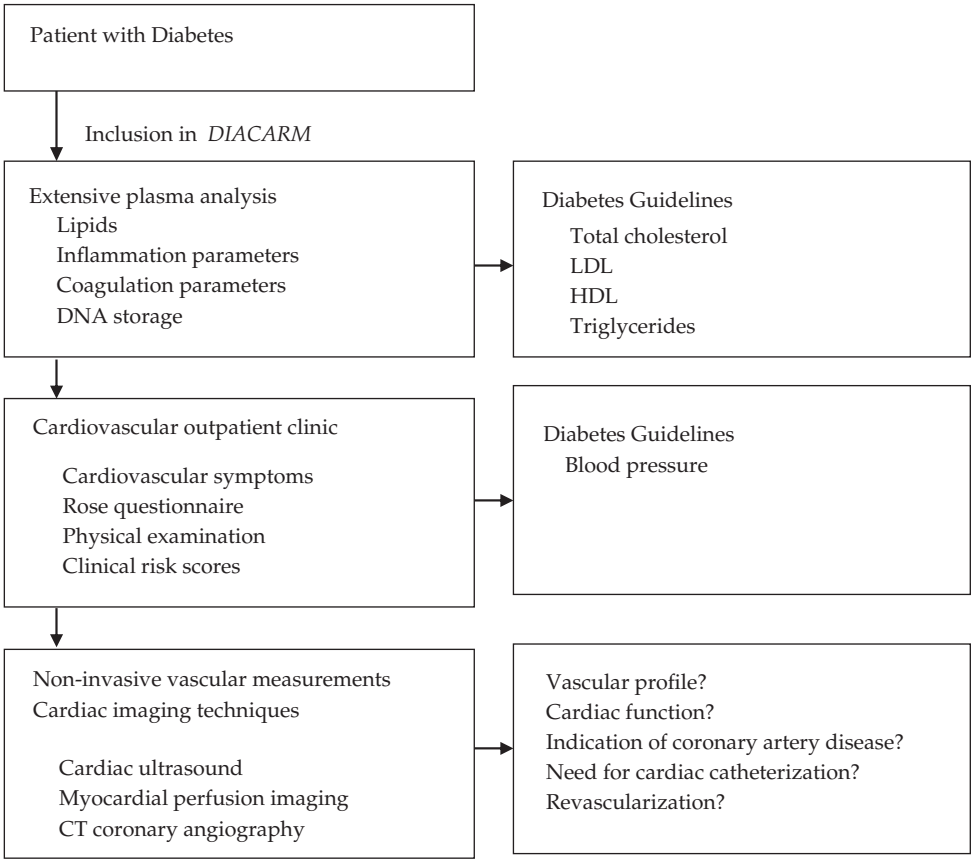
DIABETES CARDIOVASCULAR RISK MANAGEMENT: DIACARM

The diabetes cardiovascular risk management (DIACARM) project is a clinical protocol founded on a cooperation of the departments of endocrinology, cardiology, nephrology, radiology and nuclear medicine at the Leiden University Medical Center. Herein, asymptomatic patients with type 1- or type 2 diabetes, who visit the diabetes outpatient clinic are referred to the cardiology department for a cardiac risk stratification.

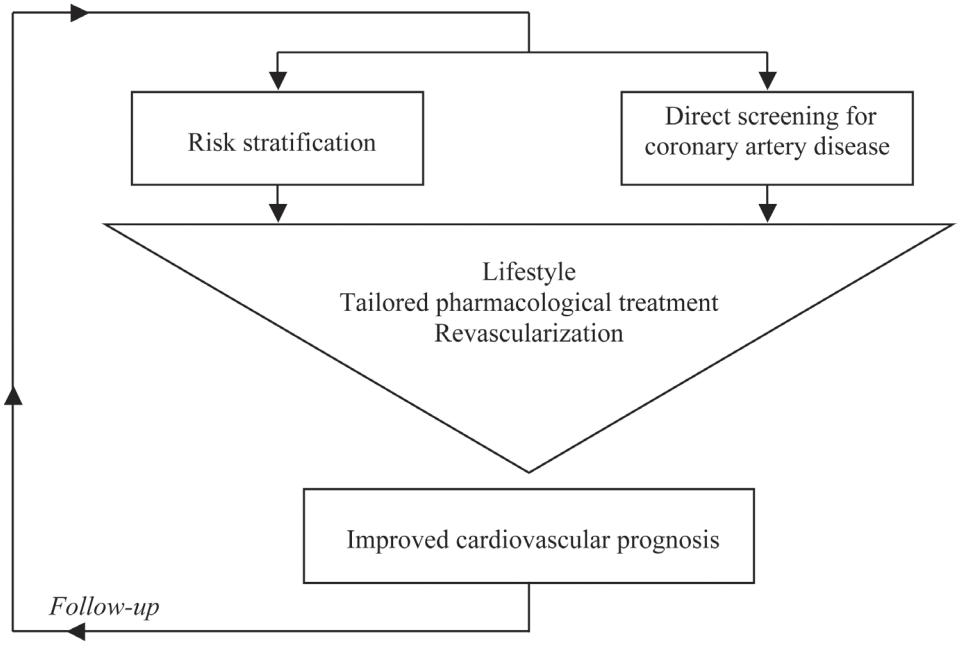


Initially, the asymptomatic status of the patients is confirmed. The medical history and presence of cardiovascular risk factors is registered. Physical examination is performed to assess blood pressure, presence of heart failure, peripheral arterial disease and/or neuropathy. Blood samples are taken, which are partly used to assess kidney function and lipid profile in the clinical setting. Additional plasma and DNA is collected and stored

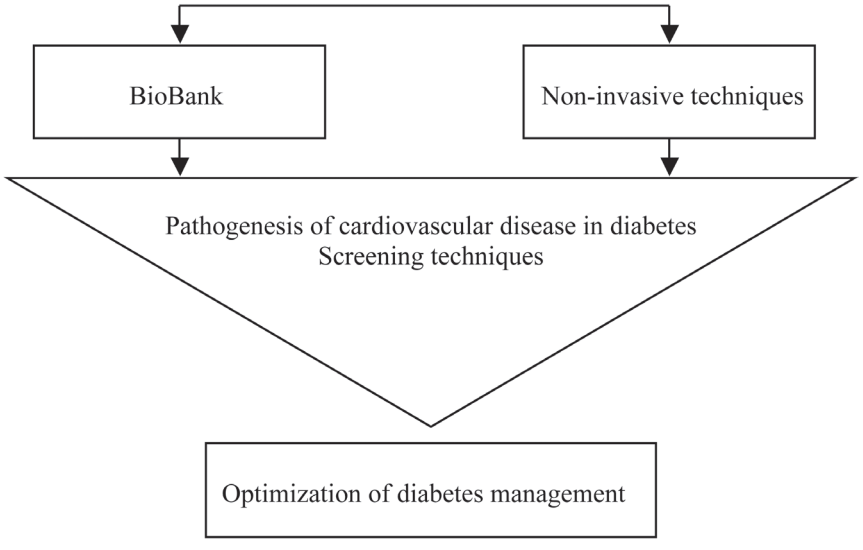
for later analysis in study setting, approved by the institutional review committee of the Leiden University Medical Center, Leiden. Non-invasive multi-slice CT angiography (CTA) of the coronaries and myocardial perfusion imaging using SPECT is performed as part of clinical work up. Concurrent non-invasive vascular measurements are performed in a study setting, also approved by the institutional review committee of the Leiden University Medical Center, Leiden. Surrogate markers of atherosclerosis comprising of CIMT and vascular stiffness parameters are assessed by ultrasound and applanation tonometry. Endothelial function is evaluated by the ultrasound assessment of the brachial flow mediated dilatation. Furthermore, a novel imaging technique, sidestream dark field imaging was applied to study the labial microcirculation at capillary level. All obtained data is prospectively recorded in a database. The database was the basis for the studies presented in this thesis. The DIACARM is an ongoing protocol and registry continues as this thesis is written.



DIACARM: Aim at individual level



DIACARM: Aim at population level



OBJECTIVE AND OUTLINE OF THE THESIS

The primary objective of the thesis is to evaluate and compare various techniques and strategies for risk stratification of CAD in asymptomatic patients with diabetes.
In Part 1 of the thesis CAD and its correlation with various parameters of metabolic syndrome are described. Furthermore the nature of CAD in diabetes is assessed. Chapter 2 describes the relation of epicardial adipose tissue with CAD as determined by CTA. In Chapter 3 the relation of adipose tissue product, adiponectin, with the parameters of CAD on CTA is evaluated. Chapter 4 describes and compares the extent, degree and morphology of CAD as assessed by CTA in asymptomatic patients with type 1- and type 2 diabetes. In Chapter 4, the relation of abnormal myocardial perfusion on SPECT in absence of epicardial obstructive CAD with endothelial dysfunction is evaluated. Part 2 of the thesis is dedicated to the risk assessment for CAD in asymptomatic patients with diabetes. A review of this topic is provided in Chapter 6. In Chapter 7 the relation of CIMT with prevalent CAD on CTA is explored. Chapter 8 further describes the relation between an increased CIMT with abnormal myocardial perfusion on SPECT. In Chapter 9, the parameters of vascular stiffness are evaluated for the prediction of abnormal myocardial perfusion on SPECT. The incremental value of coronary artery calcium scoring over micro-albuminuria for the prediction of asymptomatic myocardial ischemia on SPECT in type 1 diabetes is described in Chapter 10. In Chapter 11, risk stratification techniques, comprising the Framingham risk model, CIMT, PWV and coronary calcium scoring are compared for the identification of obstructive CAD accompanied by abnormal myocardial perfusion. Part 3 of the thesis describes a novel technique, sidestream dark field imaging, for the assessment of the microcirculation at capillary level. In Chapter 12, the sidestream dark field imaging device is validated for the assessment of the labial microcirculation parameters comprising of the capillary density and tortuosity. Chapter 13, firstly compares the microcirculation parameters in the healthy non-diabetic individuals with that in diabetic patients. Thereafter, the relation between microcirculation parameters with the presence of CAD on CTA is described in diabetic patients.

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27:1047-1053.
2. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 1997; 20:1183-1997.
3. Chaisson JL, Rabasa-Lhoret R. Prevention of type 2 diabetes: insulin resistance and beta-cell function. Diabetes. 2004; 53 Suppl 3:S34-S38.
4. Faideau B, Larger E, Lepault F, Carel JC, Boitard C. Role of beta-cells in type 1 diabetes pathogenesis. Diabetes. 2005; 54 Suppl 2:S87-S96.
5. Morrish NJ, Wang SL, Stevens LK, Fuller JH, Keen H. Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. Diabetologia. 2001; 2: S14–S21.
6. Wackers FJ, Young LH, Inzucchi SE, Chyun DA, Davey JA, Barrett EJ, Taillefer R, Wittlin SD, Heller GV, Filipchuk N, Engel S, Ratner RE, Iskandrian AE; Detection of Ischemia in Asymptomatic Diabetics Investigators. Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. Diabetes Care. 2004; 27:1954-1961.
7. Scholte AJ, Schuijf JD, Kharagjitsingh AV, Dibbets-Schneider P, Stokkel MP, Jukema JW, van der Wall EE, Bax JJ, Wackers FJ. Different manifestations of coronary artery disease by stress SPECT myocardial perfusion imaging, coronary calcium scoring, and multislice CT coronary angiography in asymptomatic patients with type 2 diabetes mellitus. J Nucl Cardiol. 2008; 15:503-509.
8. Creager MA, Lüscher TF, Cosentino F, Beckman JA. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy—part I. Circulation. 2003; 108:1527–1532.
9. Tan KC, Chow WS, Ai VH, Metz C, Bucala R, Lam KS. Advanced glycation end products and endothelial dysfunction in type 2 diabetes. Diabetes Care. 2002; 25:1055–1059.
10. Retnakaran R, Zinman B. Type 1 diabetes, hyperglycaemia, and the heart. Lancet. 2008; 371:1790-1799.
11. Silva JA, Escobar A, Collins TJ, Ramee SR, White CJ. Unstable angina. A comparison of angioscopic findings between diabetic and nondiabetic patients. Circulation. 1995; 92:1731-1736.
12. Burke AP, Kolodgie FD, Zieske A, Fowler DR, Weber DK, Varghese PJ, Farb A, Virmani R. Morphologic findings of coronary atherosclerotic plaques in diabetics: a postmortem study. Arterioscler Thromb Vasc Biol. 2004; 24:1266-1271.
13. Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med. 1998; 339:229-234.
14. Bulugahapitiya U, Siyambalapitiya S, Sithole J, Idris I. Is diabetes a coronary risk equivalent? Systematic review and meta-analysis. Diabet Med. 2009; 26:142-148.

15. Cano JF, Baena-Diez JM, Franch J, Vila J, Tello S, Sala J, Elosua R, Marrugat J; REGICOR and GEDAPS Investigators. Long-term cardiovascular risk in type 2 diabetic compared with nondiabetic first acute myocardial infarction patients: a population-based cohort study in southern Europe. Diabetes Care. 2010; 33:2004-2009.
16. Buse JB, Ginsberg HN, Bakris GL, Clark NG, Costa F, Eckel R, Fonseca V, Gerstein HC, Grundy S, Nesto RW, Pignone MP, Plutzky J, Porte D, Redberg R, Stitzel KF, Stone NJ; American Heart Association; American Diabetes Association. Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. Circulation. 2007; 115:114-126.
17. Soedamah-Muthu SS, Fuller JH, Mulnier HE, Raleigh VS, Lawrenson RA, Colhoun HM. High risk of cardiovascular disease in patients with type 1 diabetes in the U.K.: a cohort study using the general practice research database. Diabetes Care. 2006; 29:798-804.
18. Young LH, Wackers FJ, Chyun DA, Davey JA, Barrett EJ, Taillefer R, Heller GV, Iskandrian AE, Wittlin SD, Filipchuk N, Ratner RE, Inzucchi SE; DIAD Investigators. Cardiac outcomes after screening for asymptomatic coronary artery disease in patients with type 2 diabetes: the DIAD study: a randomized controlled trial. JAMA. 2009; 301:1547-1555.
19. Coleman RL, Stevens RJ, Retnakaran R, Holman RR. Framingham, SCORE, and DECODE risk equations do not provide reliable cardiovascular risk estimates in type 2 diabetes. Diabetes Care. 2007; 30:1292-1293.
20. Lee CD, Folsom AR, Pankow JS, Brancati FL, Atherosclerosis Risk in Communities (ARIC) Study Investigators. Cardiovascular events in diabetic and nondiabetic adults with or without history of myocardial infarction. Circulation. 2004; 109:855–860.
21. Hatsuda S, Shoji T, Shinohara K, Kimoto E, Mori K, Fukumoto S, Koyama H, Emoto M, Nishizawa Y. Regional arterial stiffness associated with ischemic heart disease in type 2 diabetes mellitus. J Atheroscler Thromb. 2006; 13:114-121.
22. Bernard S, Sérusclat A, Targe F, Charrière S, Roth O, Beaune J, Berthezène F, Moulin P. Incremental predictive value of carotid ultrasonography in the assessment of coronary risk in a cohort of asymptomatic type 2 diabetic subjects. Diabetes Care. 2005; 28:1158–1162.
23. Yamasaki Y, Kodama M, Nishizawa H, Sakamoto K, Matsuhisa M, Kajimoto Y, Kosugi K, Shimizu Y, Kawamori R, Hori M. Carotid intima–media thickness in Japanese type 2 diabetic subjects: predictors of progression and relationship with incident coronary heart disease. Diabetes Care. 2000; 23:1310–1315.

PART I

Coronary artery disease in adiposity and in diabetes

CHAPTER 2

Relation of Epicardial Adipose Tissue to Coronary Atherosclerosis

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Am J Cardiol. 2008;102:1602-1607.

ABSTRACT

Adipose tissue surrounding the coronary arteries has been suggested to induce development of atherosclerosis. We explored the relation between epicardial adipose tissue (EAT) volume and coronary atherosclerosis using multislice computed tomography. The study population consisted of 190 patients who had undergone multislice computed tomographic coronary angiography. Coronary artery calcium score was assessed. In addition, patients were classified as having (1) no atherosclerosis, (2) nonobstructive atherosclerosis (luminal narrowing <50%), (3) obstructive atherosclerosis (luminal narrowing ≥50%) in a single vessel or (4) obstructive atherosclerosis in the left main and/or multiple vessels. Cross-sectional tomographic cardiac slices (3.00 mm thickness, range 35-40 slices per heart) were traced semi-automatically from the border of EAT below the apex to a point at the center of the left atrium. Tissue ranging from -250 HU to -30 HU was assigned as EAT. EAT volume within the traced area was then automatically quantified. Mean EAT volume was 84 ± 41 ml. Patients with a coronary artery calcium score >10 had significantly higher average EAT volume (100 ml ± 40) compared to patients with calcium scores ≤10 (59 ml ± 27), (P<0.001). Sensitivity and specificity for prediction of a calcium score >10 were 77% and 70% with a cut-off EAT value of 73 ml. In patients with normal coronaries mean EAT volume (63 ml ±31) was significantly lower than in patients with atherosclerosis (99 ml ± 40), (P<0.001). Using a cut-off EAT volume of 75 ml, the sensitivity and specificity for presence of atherosclerosis were 72% and 70%. Interestingly, the quantity of EAT did not significantly increase with increasing extent or severity of atherosclerosis. After adjustments for risk factors EAT volume remained a significant predictor of coronary atherosclerosis (P=0.001). In conclusion, a significant relation was shown between EAT volume and the presence of coronary atherosclerosis. Quantification of EAT may be useful to identify patients at risk for coronary artery disease.

INTRODUCTION

The relation between abdominal adipose tissue and predisposition to coronary artery disease (CAD) has been well established. Recent data suggest that epicardial adipose tissue (EAT) may also be associated with CAD. In particular, a local inflammatory effect has been suggested since EAT directly surrounds the coronary arteries.[1,2] To date, several modalities have been applied to quantify EAT. Ahn et al observed a good correlation between EAT thickness as determined on echocardiography and the presence of angiographic CAD.[3] However, assessment of EAT by echocardiography is limited to measurements of thickness of the adipose tissue on the free wall of the right ventricle. As a result, contradictory results have been reported by other investigators.[4] multislice computed tomography may provide a more accurate and reproducible quantification of EAT due to its higher spatial resolution. In addition the technique allows quantification of peri-coronary fat thickness and total EAT volume.[5] Reproducibility of volumetric EAT measurements have shown to be superior to thickness measurements.[5] Two recent studies have evaluated the association between EAT assessed by multislice computed tomography and coronary artery calcium (CAC) scores. A positive association was observed between EAT quantity and coronary calcium.[6,7] However, the direct relationship between EAT quantity assessed by MSCT and the presence of coronary atherosclerosis has not been analyzed thus far. This study explores the relation between EAT volume assessed by MSCT and the presence of coronary atherosclerosis. The association between EAT volume and CAC scores and with the presence and degree of coronary atherosclerosis was determined.

METHODS

The study population consisted of 190 patients who were clinically referred for non-invasive multislice computed tomographic (MSCT) coronary angiography for the evaluation of CAD. Standard exclusion criteria for MSCT coronary angiography were ventricular and supraventricular arrhythmia and contraindications for the use of iodinated contrast media. In addition patients with poor image quality (n=6) and previous cardiac surgery were excluded from the study.

MSCT coronary angiography was performed with a 64-slice MSCT scanner (Toshiba Medical Systems, Japan and Lightspeed VR 64, General Electrics, Milwaukee, MI, USA). If the heart rate was ≥ 65 beats per minute, oral beta-blockers (metoprolol 50 mg or 100 mg) were provided 1 hour preceding the scan, if tolerated. Initially, a triggered CAC scan was obtained. Hereafter, MSCT angiography was performed using the following parameters: collimation 64x0.5 mm or 64x0.625, tube rotation time 350, 400, 450 or 500 ms depending on the heart rate, tube current 300, 350 or 600 mA, tube voltage 120 kV. Non-ionic contrast material was administered in the antecubital vein in an amount of

80–100 ml, depending on the total scan time, and a flow rate of 5 ml/s, followed by a saline solution flush of 50 ml. Automated bolus-tracking in the aortic root was used for the timing of the scan. Images were acquired with simultaneous electrocardiogram registration during a single breath hold of approximately 10 seconds. Images were reconstructed in the cardiac phase showing least motion artifacts. In general, the end-diastolic phase was used. However, additional reconstructions were made throughout the entire cardiac cycle if necessary. Reconstructed images were transferred to remote workstations (Advantage, GE Healthcare, USA; and Vitrea 2, Vital Images, USA) for post-processing.

The CAC score was assessed with the application of dedicated software (Vitrea2, Vital Images, USA). Coronary calcium was identified as a dense area in the coronary artery exceeding the threshold of 130 Hounsfield units (HU). An overall Agatston score was registered for each patient.

All coronary angiograms were evaluated by 2 experienced observers. The presence of coronary atherosclerosis was assessed by scrolling through axial images, followed by visual assessment of curved multiplanar reconstructions in at least 2 orthogonal planes. Patients were classified as having 1. no atherosclerosis 2. non-obstructive atherosclerosis (luminal narrowing <50% in diameter) 3. obstructive atherosclerosis (luminal narrowing ≥50%) in a single vessel or 4. obstructive atherosclerosis in the left main and/or multiple vessels.

EAT was quantified using dedicated software (Advantage, GE Healthcare, USA) using the same ECG-gated MSCT reconstructions. EAT was defined as the adipose tissue between the surface of myocardium and the epicardium. Short axis reconstruction slices (3 mm thickness) were created semi-automatically ranging from the surface of epicardium at the apex to a cut-off point at the center of the left atrium for each patient. The number of slices ranged between 35 and 40 per heart. The outer border of epicardium was traced manually on each slice. The total volume within the selected areas (in ml) was produced automatically by the computer software program by adding up the EAT areas and taking the slice thickness into account. Hounsfield Units ranging from -250 to -30 were assigned to isolate adipose tissue within the total selected volume. Adipose tissue within the selected volume was then automatically quantified by the software (Figure 1).

Firstly, the relationship between CAC score and EAT volume was analyzed. The study population was divided into four groups according to CAC scores: patients with a CAC score 0-10, CAC score 11-100, CAC score 101-400 and those with a CAC score >400. Average EAT volume and standard deviation were calculated in each group. The independent T-test was used to assess the difference in mean EAT volume between the groups. ROC curve analysis was used to select a cut-off value for EAT volume. Consequently sensitivity and specificity for predicting a CAC score >10 were calculated.

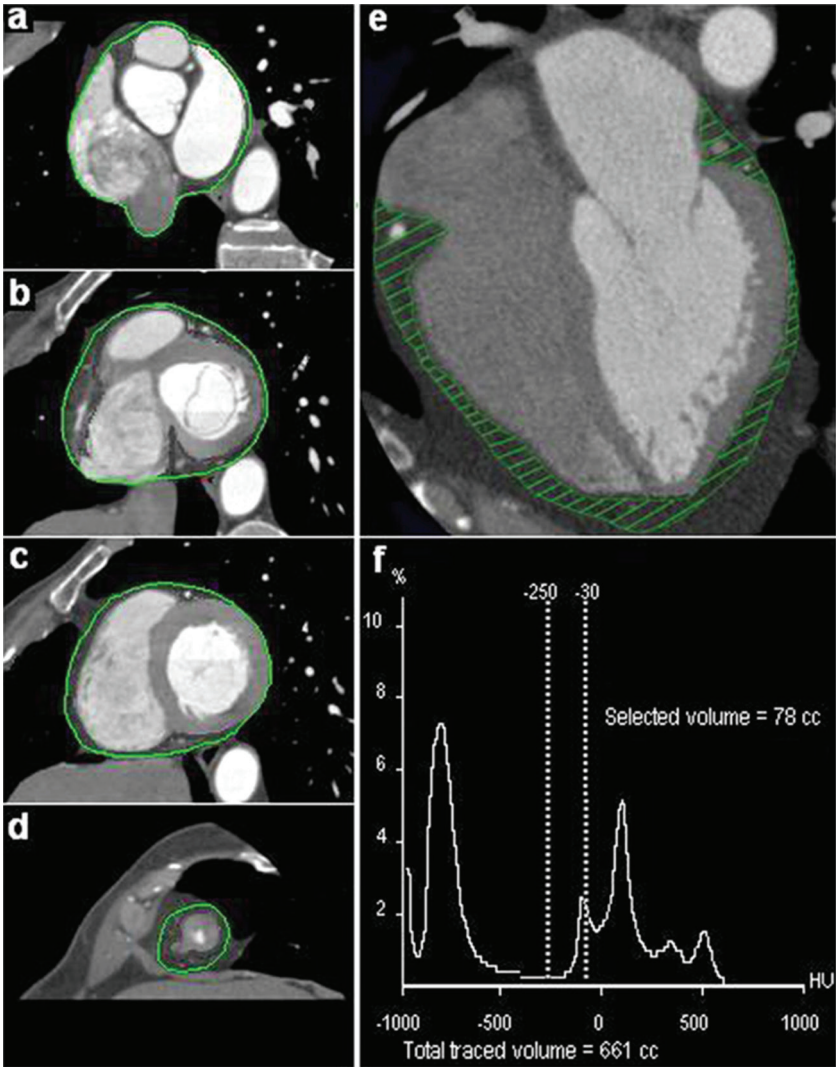

Figure 1. Quantification of EAT using dedicated software. The outer border of epicardium was traced manually on short axis slices from the surface of epicardium at the apex to a cut-off point at the center of the left atrium. (*a to d*) Hounsfield Units (HU) ranging from -250 to -30 were assigned to isolate adipose tissue within the total selected volume. Adipose tissue within the selected volume was then automatically identified (*e*) and quantified (*f*).

Table 1. Characteristics of the study population (n=190)

Age (years)	56 ± 12
Men	104 (55%)
Body Mass Index (kg/m2)	27 ± 4
Smokers	51 (27%)
Family history of Coronary Artery Disease	87 (46%)
Hypercholesterolemia*	143 (75%)
Hypertension**	134 (71%)
Diabetes Mellitus	20 (11%)

Data are averages ± standard deviation or numbers of patients (%).

* Total cholesterol level >5.0 mmol/L or use of cholesterol lowering medication.

** Blood pressure > 140/90 mmHg or treatment with antihypertensive medication.

To evaluate the relationship between coronary atherosclerosis and EAT volume, the study population was classified into four groups according to the results of the non-invasive MSCT angiography: patients with normal coronary arteries, patients with non-obstructive atherosclerosis, patients with obstructive atherosclerosis in a single coronary artery and patients with obstructive atherosclerosis in the left main coronary artery and/or multiple vessels. Average EAT volume and standard deviation were calculated for each group, and the independent T-test was applied to evaluate the difference in mean EAT volume between the groups. Using ROC analysis a cut-off value was chosen for EAT volume. Subsequently sensitivity and specificity for predicting coronary atherosclerosis were calculated. Univariate analysis of baseline characteristics was performed to identify potential predictors of coronary atherosclerosis. Hazard ratios were calculated with 95% confidence interval as an estimate of the risk associated with coronary atherosclerosis. To determine the independent predictors of atherosclerosis, multivariate analysis of risk factors with $p \leq 0.05$ in the univariate analysis was performed which corrected for the baseline characteristics with $p \leq 0.05$ in the univariate analysis.

RESULTS

Patient characteristics are provided in Table 1. Mean age of the study population was 56 years (± 12). The majority of patients were male (55%).

Mean CAC score was 358 ± 906 in the total population. CAC was absent or ≤10 in 71 patients (37%) and >10 in 119 patients (63%). Within this group, 45 patients (24%) had a CAC score in the range 11-100, 33 patients (17%) had a CAC score in the range 101-400 and 41 patients (22%) had a CAC score >400. MSCT coronary angiography showed normal coronaries in 76 patients (40%). A total of 45 patients (24%) had non-obstructive atherosclerosis (luminal narrowing <50%), and obstructive coronary atherosclerosis

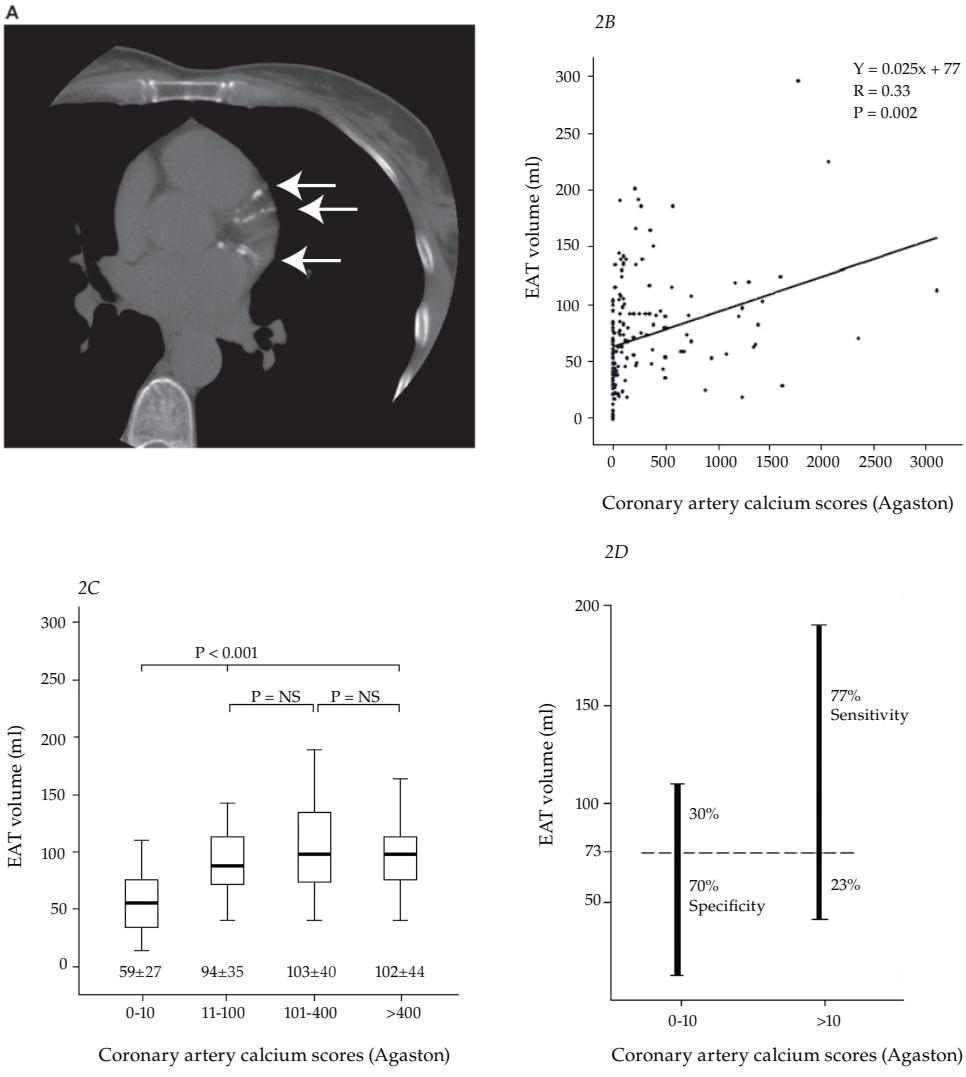

Figure 2. (*A*) Example of coronary artery calcium scoring with MSCT. Diffuse calcification can be observed in the left anterior ascending coronary artery, first diagonal and left circumflex coronary artery (arrows). (*B*) Pearson correlation showed a significant but only moderate correlation between EAT volume (milliliters) and CAC scores (r=0.33, p=0.002). Mean EAT volume in patients with a CAC score 0-10 and in those with a CAC score >10. (*C*) Mean EAT volume was significantly higher in patients with a CAC score >10. However, it did not significantly differ between patients with CAC scores 11-100, 101-400 and >400. (*D*) Sensitivity and specificity of EAT volume with a cut-off value of 73 ml for predicting a CAC score >10 were 77% and 70%, respectively.

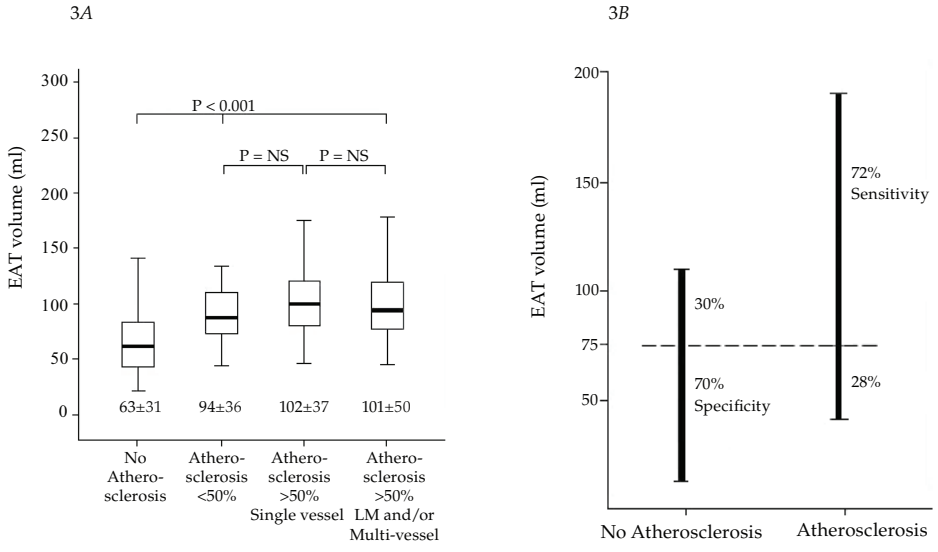

Figure 3. Relationship between EAT volume and coronary atherosclerosis. (*A*) Mean EAT volume in patients with no atherosclerosis, non-obstructive atherosclerosis, obstructive single vessel atherosclerosis and obstructive left main and/or multi-vessel atherosclerosis. (*B*) Sensitivity and specificity of EAT volume with a cut-off value of 75 ml for predicting coronary atherosclerosis were 72% and 70%, respectively.

Table 2. Predictors of Atherosclerosis

	Univariate Analysis		Multivariate Analysis	
Characteristic	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
EAT volume	1.03 (1.02-1.05)	<0.001	1.03 (1.01-1.05)	0.001
Age	1.10 (1.06-1.13)	<0.001	1.05 (1.00-1.09)	0.03
Hypertension	0.09 (0.04-0.22)	<0.001	0.12 (0.03-0.45)	0.002
Hypercholesterolemia	0.09 (0.03-0.25)	<0.001	0.06 (0.01-0.24)	<0.001
Body Mass Index (kg/m^2)	1.11 (1.03-1.20)	0.01	1.04 (0.91-1.18)	0.56
Diabetes Mellitus	1.58 (0.62-4.00)	0.34	-	-
Smoking	0.79 (0.40-1.56)	0.46	-	-
Family history of cardiovascular disease	1.18 (0.63-2.19)	0.61	-	-
Male gender	1.06 (0.59-1.89)	0.86	-	-

CI= confidence interval, HR= hazard ratio.

defined as luminal narrowing ≥ 50% was observed in 69 patients (36%). Obstructive single vessel atherosclerosis was observed in 43 patients (22%) and 26 patients (14%) had obstructive left main and/or multi-vessel atherosclerosis.

EAT volume ranged between 15 ml and 267 ml with an average of 84 ± 41 ml. EAT volume showed a tendency to increase with coronary calcium (r=0.33, p=0.002) (Fig 2A). Indeed, average EAT volume was significantly higher in patients with a CAC score >10 (100 ml ± 40) as compared to patients with calcium scores ≤10 (59 ml ± 27), (P<0.001) (Fig 2B). Mean EAT volume did not differ significantly between patients with a CAC score 11-100 (94 ml ±35), CAC score 101-400 (103 ml ± 40) and those with a CAC score >400 (102 ml ± 44) (Figure 2B). Using ROC analysis a cut-off value of 73 ml was identified for EAT volume. This cut-off value yielded a sensitivity and specificity of respectively 77% and 70% for predicting a calcium score >10 (Fig 2C).

In patients with normal coronaries mean EAT volume (63 ml ± 31) was significantly lower than in those with atherosclerosis (99 ml ± 40), (P<0.001) (Fig 3A). However, quantity of EAT did not significantly differ between patients with non-obstructive and obstructive coronary atherosclerosis (P= 0.29). In patients with obstructive atherosclerosis no significant difference was observed in mean EAT volume between patients with single vessel atherosclerosis (102 ml ± 37) as compared to those with left main and/or multi-vessel atherosclerosis (101 ml ± 50) (P=0.95) (Fig 3A). ROC analysis resulted in a sensitivity and specificity of respectively 72% and 70% with an EAT cut-off volume of 77 ml, for predicting coronary atherosclerosis (Fig 3B).

In a univariate model, age, EAT volume, BMI, hypercholesterolemia and hypertension were shown to be predictors of coronary atherosclerosis (Table 2). After adjustment for age, BMI, hypertension and hypercholesterolemia in a multivariate model, EAT volume remained a significant predictor of coronary atherosclerosis (Table 2).

DISCUSSION

In the present study a relation was shown between EAT volume and the presence of CAC and atherosclerosis. Mean EAT volume was significantly higher in patients with CAC and/or coronary atherosclerosis on MSCT angiography in comparison to those with normal coronaries. Importantly, EAT volume was shown to be an independent predictor of coronary atherosclerosis.

Most previous studies which have examined the relationship between CAD and EAT have used echocardiography for the quantification of EAT. Thus far, results of these studies have been controversial. Ahn et al studied the association between thickness of EAT on the free wall of the right ventricle and CAD in patients who underwent conventional coronary angiography due to chest pain.[3] A positive association was found between thickness of EAT and presence of significant coronary stenosis (luminal narrowing

≥50%) and with the number of coronary arteries with significant stenosis. In contrast, analysis by Chaowalit and colleagues in 139 patients who were referred for conventional coronary angiography did not show a significant correlation between EAT thickness and the number of atherosclerotic coronary segments.[4] The discrepancy between these findings may be due to the limitations of echocardiography in quantification of EAT including inaccuracy of measurements due to lower resolution (especially in adiposity) while also difficulties in the delineation of EAT and pericardial fat may be frequently encountered.[8] In addition, echocardiographic estimation of EAT quantity is limited to the measurement of the EAT thickness on the free wall of the right ventricle, whereas it has been shown that the pattern of distribution of EAT surrounding the myocardium may vary significantly amongst individuals.[9] It is therefore likely that EAT thickness on the free wall of the right ventricle may not always be a reliable and accurate representative of total EAT quantity.

Multislice computed tomography provides an accurate and reproducible quantification of EAT due to its high temporal and spatial resolution. Quantification of both pericoronary fat thickness and total EAT volume are possible.[5] Reproducibility of volumetric EAT measurements has been shown to be superior to thickness measurements.[5] Two previous studies have analyzed the association between EAT assessed by MSCT using CAC as a marker of atherosclerosis. De Vos et al. studied the relation between pericoronary fat thickness assessed by MSCT and CAC scores in a female population and found a graded relationship.[7] Similarly, in a substudy of the Framingham Heart investigation (n=1155 individuals) EAT volume was shown to be associated with CAC.[6] The results of the current study are in line with these investigations. Additionally, in the current study the presence and extent of coronary atherosclerosis was directly assessed by MSCT angiography and studied in relation to the quantity of EAT volume. We observed significantly higher mean volume of EAT in patients with coronary calcifications. Importantly mean EAT volume was also significantly higher in patients with coronary atherosclerosis as compared to those with normal coronaries. However, in patients with atherosclerosis no significant relation was observed between mean EAT volume and the extent or degree of coronary atherosclerosis.

In our current study EAT volume was shown to be an independent predictor of coronary atherosclerosis in addition to the established cardiovascular risk factors. Of interest, EAT volume remained a significant predictor even after correction for BMI suggesting that EAT volume is not purely a reflection of BMI in the predisposition of atherosclerosis. Rather, EAT may stimulate atherosclerosis due to a direct local effect on the coronaries. This notion is supported by several studies exploring the inflammatory responses of EAT. Mazurek et al. compared the local concentration and expression of inflammatory markers between EAT and subcutaneous fat in patients who underwent

coronary artery bypass graft surgery.[1] Increased expressions and protein levels of interleukin-1 beta, interleukin-6 and tumor necrosis factor-alpha mRNA were observed in the EAT as compared to subcutaneous fat. Thus, inflammatory EAT response was not associated with plasma inflammatory biomarkers. In another study by Iacobellis and colleagues suppressed levels of the anti-inflammatory adiponectin were observed in the EAT samples of high risk cardiac patients.[2] However, it could also be argued that the inflammatory responses observed in the above studies may be a result of coronary atherosclerosis rather than a cause. Accordingly, plaque rupture in the coronary arteries and regional myocardial ischemia have been shown to trigger an inflammatory reaction in the adjacent adipose tissue stores.[10,11] Thus, the precise pathophysiological interaction between EAT and the coronary wall with respect to development of coronary atherosclerosis remains to be further elucidated.

Several limitations need to be acknowledged. The present study was performed in a heterogeneous study population. The current analysis was restricted to evaluation of the association between coronary atherosclerosis and EAT quantity. The proatherogenic process, which relates EAT and predisposition to atherosclerosis, was not investigated. Furthermore, no follow-up data were available. Indeed, whether EAT quantity may have a prognostic value needs to be evaluated in prospective follow up studies.

REFERENCES

1. Mazurek T, Zhang L, Zalewski A, Mannion JD, Diehl JT, Arafat H, Sarov-Blat L, O'Brien S, Keiper EA, Johnson AG, Martin J, Goldstein BJ, Shi Y. Human epicardial adipose tissue is a source of inflammatory mediators. *Circulation* 2003; 108: 2460-2466.
2. Iacobellis G, Pistilli D, Gucciardo M, Leonetti F, Miraldi F, Brancaccio G, Gallo P, di Gioia CR. Adiponectin expression in human epicardial adipose tissue in vivo is lower in patients with coronary artery disease. *Cytokine* 2005; 29: 251-255.
3. Ahn SG, Lim HS, Joe DY, Kang SJ, Choi BJ, Choi SY, Yoon MH, Hwang GS, Tahk SJ, Shin JH. Relationship of epicardial adipose tissue by echocardiography to coronary artery disease. *Heart* 2008; 94:e7.
4. Chaowalit N, Somers VK, Pellikka PA, Rihal CS, Lopez-Jimenez F. Subepicardial adipose tissue and the presence and severity of coronary artery disease. *Atherosclerosis* 2006; 186: 354-359.
5. Gorter PM, van Lindert AS, de Vos AM, Meijs MF, van der Graaf Y, Doevendans PA, Prokop M, Visseren FL. Quantification of epicardial and peri-coronary fat using cardiac computed tomography; reproducibility and relation with obesity and metabolic syndrome in patients suspected of coronary artery disease. *Atherosclerosis* 2008; 197: 896-903.
6. Rosito GA, Massaro JM, Hoffmann U, Ruberg FL, Mahabadi AA, Vasan RS, O'Donnell CJ, Fox CS. Pericardial fat, visceral abdominal fat, cardiovascular disease risk factors, and vascular calcification in a community-based sample: the Framingham Heart Study. *Circulation* 2008; 117: 605-613.
7. de Vos AM, Prokop M, Roos CJ, Meijs MF, van der Schouw YT, Rutten A, Gorter PM, Cramer MJ, Doevendans PA, Rensing BJ, Bartelink ML, Velthuis BK. Peri-coronary epicardial adipose tissue is related to cardiovascular risk factors and coronary artery calcification in post-menopausal women. *Eur Heart J* 2008; 29: 777-783.
8. Kanna B, Osorio F, Dharmarajan L. Pericardial fat mimicking pericardial effusion on two-dimensional echocardiography. *Echocardiography* 2006; 23: 400-402.
9. Abbara S, Desai JC, Cury RC, Butler J, Nieman K, Reddy V. Mapping epicardial fat with multi-detector computed tomography to facilitate percutaneous transepicardial arrhythmia ablation. Eur J Radiol. 2006; Epub 57: 417-422.
10. Kalra DK, Zhu X, Ramchandani MK, Lawrie G, Reardon MJ, Lee-Jackson D, Winters WL, Sivasubramanian N, Mann DL, Zoghbi WA. Increased myocardial gene expression of tumor necrosis factor-alpha and nitric oxide synthase-2: a potential mechanism for depressed myocardial function in hibernating myocardium in humans. Circulation 2002; 105: 1537-1540.
11. Moreno PR, Purushothaman KR, Fuster V, O'Connor WN. Intimomedial interface damage and adventitial inflammation is increased beneath disrupted atherosclerosis in the aorta: implications for plaque vulnerability. *Circulation* 2002; 105: 2504-2511.

CHAPTER 3

Reduced Plasma Adiponectin is Associated with Extent, Degree and Morphology of Coronary Artery Disease in Asymptomatic Patients with Type 2 Diabetes

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Submitted.

ABSTRACT

Objective

Reduced plasma adiponectin has been linked to coronary artery disease (CAD). However, little is known concerning this relation in type 2 diabetes. We explored the relation between plasma adiponectin and the parameters of coronary atherosclerosis in asymptomatic patients with type 2 diabetes.

Methods

Prospectively, multi-slice CT angiography (CTA) of the coronaries was performed in 103 asymptomatic patients with type 2 diabetes. The following parameters of atherosclerosis were assessed: presence of any atherosclerosis and obstructive atherosclerosis, atherosclerotic plaque burden (number of plaques), and plaque phenotype (number of non-calcified, mixed, calcified). Parameters of atherosclerosis were studied in relation to traditional cardiovascular risk factors, high sensitivity C-reactive protein and plasma adiponectin.

Results

An independent inverse relation was observed between plasma adiponectin and the presence of any atherosclerosis (P=0.02) and obstructive atherosclerosis (P=0.003). Accordingly, an adiponectin cut-off value of <4.5 mcg/ml resulted in a sensitivity and specificity of 80% and 71% for predicting obstructive atherosclerosis. An independent inverse relation was also observed between plasma adiponectin and the atherosclerotic plaque burden (P=0.02). Further analysis demonstrated low plasma adiponectin to be independently associated with the quantity of non-calcified (P=0.04), but not with mixed or calcified plaques.

Conclusion

Low plasma adiponectin was independently related with the parameters of coronary atherosclerosis in asymptomatic patients with type 2 diabetes. A predominant association was observed with the presence of non-calcified plaques, which have been associated with unstable disease. Assessment of plasma adiponectin conveys the potential to augment risk for CAD in asymptomatic patients with type 2 diabetes.

INTRODUCTION

Early identification of patients prone to develop coronary artery disease (CAD) and prompt initiation of appropriate therapy has become a main focus in the last decades. In consequence, prognosis of CAD has improved in the general population [1]. However, in patients with diabetes, excess CAD mortality and morbidity persist despite preventive guidelines [1]. Improved ability to gauge risk in the individual patient with diabetes, may motivate physicians and patients to adhere preventive therapy, and define those who may benefit from more aggressive risk-reduction strategies and further screening for CAD.

Nevertheless, the optimal approach for the identification of the high risk patient with diabetes remains unclear. In contrast with the general population, risk assessment methods based on the traditional clinical cardiovascular risk factors have shown limited incremental value in diabetes [2,3]. As a result, the use of surrogate markers of atherosclerosis and novel serum biomarkers has gained recent attention for this purpose [4,5]. Validated novel serum biomarkers may convey the additional advantage of reflecting on an individual's predisposition to develop CAD in an early stage, prior to evident atherosclerosis, enabling successful preventive therapy.

The serum biomarker adiponectin is the most abundant adipocytokine secreted by the adipose tissue cells [6]. Synthesis of adiponectin is reduced in obesity, insulin resistance, and type 2 diabetes [6,7]. Low levels of serum adiponectin have also been related to the presence of atherosclerosis [6]. In line with these observations, genetic and experimental studies suggest adiponectin to have an array of anti-atherosclerotic effects [8]. However, based on existing epidemiological data in the general population, the prognostic value of adiponectin remains controversial [9-11]. Interestingly, cross-sectional studies in general patient populations with manifest CAD suggest adiponectin to be related with atherosclerosis only below a certain serum threshold [12,13]. Hence, adiponectin may be most strongly related with CAD in the presence of type 2 diabetes where low ranges of the biomarker are reported [14]. Thus far, only limited information is available on the association of plasma adiponectin and CAD in patients with type 2 diabetes [14-16].

In the current study, we explored the relation between plasma adiponectin and parameters of coronary atherosclerosis as assessed by multi-slice CT, in asymptomatic patients with type 2 diabetes. The association between plasma adiponectin and the presence of any atherosclerosis and obstructive atherosclerosis, atherosclerotic plaque burden, and plaque phenotype was determined.

METHODS

Study Design and Patient Characteristics

The study population comprised of 103 asymptomatic patients with type 2 diabetes. Patients were included prospectively from an ongoing registry at the diabetes outpatient clinic. Diagnosis and classification of diabetes was performed using the American Diabetes Association criteria [17]. Accordingly, presence of type 2 diabetes (prominent insulin resistance) was distinguished from primary insulinopenia and immune destruction of beta pancreas cells by determining plasma levels of C-peptide and auto-antibodies to islet cells, insulin and glutamic-acid-decarboxylase. Consecutive patients with type 2 diabetes were referred to the cardiology outpatient clinic for risk stratification and cardiovascular screening. Anginal symptoms were ruled out using a self-completed questionnaire for encountered chest pain [18]. Patients underwent clinical and laboratory evaluation. Non-invasive multi-slice CT angiography (CTA), of the coronaries was performed as part of clinical work up. Concurrently, plasma was collected and stored for later analysis in a study setting, approved by the institutional review committee of the Leiden University Medical Center, Leiden. All patients gave written consent. Patients not eligible for CTA due to arrhythmia or contraindications for the use of iodinated contrast media were excluded.

Cardiovascular risk factors

Presence of cardiovascular risk factors was defined as: 1. smoking (current smoking or smoking in the last 2 years), 2. positive family history of CAD (CAD in first degree family members <55 years in men or <65 years of age in women), 3. body mass index (BMI) as kg/m^2, 4. hypertension (blood pressure >140/90 mmHg or treatment with antihypertensive medication), 5. hypercholesterolemia (total cholesterol level >5.0 mmol/L or use of lipid lowering medication), and 5. glycosylated hemoglobin A1c (HbA1c) as a measure of glycemic control, 6. micro-albuminuria (urine albumin/creatinine ratio ≥3.5 mg/mmol).

MSCT data acquisition

Imaging was performed using a 64-slice multi-slice CT scanner (Aquilion64, Toshiba Medical Systems, Japan). If necessary and tolerated, oral beta-blockers (metoprolol 50 mg or 100 mg) were provided 1 hour preceding the scan to achieve a heart rate <65 beats per minute. First, a non-enhanced prospective electrocardiographically gated scan, triggered at 75% of the R-R interval with 4 x 3.0 mm collimation was obtained to determine the start and end position of the helical scan.

Second, CTA was performed using the following parameters: collimation 64 x 0.5 mm, tube rotation time 400, 450 or 500 ms depending on the heart rate, tube current 300 or 350 mA, tube voltage 120 kV. Non-ionic contrast material was administered in the antecubital vein at a flow rate of 5 ml/L and the amount of 90–105 ml (depending on the total scan time), followed by 50 ml of saline solution flush. Automated bolus-tracking in the aortic root was applied for the timing of the scan. Images were acquired with simultaneous ECG registration during a single breath hold of approximately 10 seconds. Segmental reconstruction algorithm was applied to generate a single image from the data of one, two or three consecutive heartbeats. Images were reconstructed in the cardiac phase showing least motion artifacts. In general, the end-diastolic phase was used. However, additional reconstructions were made throughout the entire cardiac cycle if necessary to improve image quality. Subsequently, the images were transferred to a remote workstation (Vitrea 2, Vital Images, Minnetonka, USA) for post-processing.

CTA data analysis

All CTA's were interpreted by two experienced observers blinded to patient characteristics. Discrepancies in interpretation were immediately resolved by consensus. The presence of coronary atherosclerosis was evaluated by scrolling through axial images, followed by visual assessment of curved multiplanar reconstructions in at least two orthogonal planes. Coronary plaques were defined as structures of >1 mm^2 within and/or adjacent to the coronary artery lumen, which could be vividly discriminated from the vessel lumen and the surrounding pericardial tissue [19].

Initially, the presence of any atherosclerosis (≥1 plaque in the coronary tree), and that of obstructive atherosclerosis (luminal narrowing ≥50%) were evaluated in each patient. Thereafter, plaque burden was obtained by determining the total number of atherosclerotic plaques and obstructive plaques per patient [19].

Finally, plaques were classified according to phenotype: 1. non-calcified plaques (plaques with lower density than contrast-enhanced lumen), 2. calcified plaques (plaques with higher density than contrast-enhanced lumen), and 3. mixed plaques (plaques with components of low- and high density plaques) [19].

Laboratory Analysis

From all patients, blood and urine were collected after fasting overnight. Plasma total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, HbA1c and urine albumin/creatinine ratio were determined in the hospital laboratory, following clinical evaluation.

Blood samples collected for later analysis of high sensitivity C-reactive protein (hsCRP) and adiponectin in study setting were centrifuged. Separated plasma was stored at

-80ºC until assayed. All subsequent laboratory analyses were performed blind to patient characteristics.
Plasma levels of hsCRP were determined using the Tina-quant immunoassay (Roche Diagnostics, United Kingdom) by a Cobas Integra 800 analyzer. The method has a detection limit of 0.15 mg/L and a functional sensitivity of 0.3 mg/L. The inter-assay and intra-assay coefficients of variation are <3%.
Plasma adiponectin was measured with a commercially available radioimmunoassay (Linco Research, Inc, St Charles, Missouri, USA). The sensitivity cut-off was 1 ng/ml. The intra-assay and the inter-assay coefficients of variability were 6.21% and 6.90%, for mid-range concentrations of adiponectin respectively. Plasma adiponectin results are reported in micrograms per millimeter.

Statistical Analysis
Variables were expressed as means ± standard deviation, medians (lower quartile – upper quartile) or as numbers (percentages) if categorical.
First, the correlation between plasma adiponectin and traditional cardiovascular risk factors as well as hsCRP was determined using the Pearsons correlation.
Second, the relations between plasma adiponectin and the presence and degree of coronary atherosclerosis were evaluated. For this purpose the median plasma adiponectin levels were determined and compared in patients with no atherosclerosis, non-obstructive atherosclerosis and obstructive coronary atherosclerosis. The Mann-Whitney U test was applied to evaluate the difference in plasma adiponectin levels between the groups.
Thereafter, the potential predictors of the presence of any coronary atherosclerosis and obstructive coronary atherosclerosis on CTA were studied in univariate logistic regression models. All baseline traditional cardiovascular risk factors, as well as plasma hsCRP and adiponectin levels were included in the analyses. Variables with a P value <0.05 were included in a multivariate logistic regression model to identify the independent predictors.
Similarly, the predictors of extent of atherosclerosis represented by the number of atherosclerotic plaques, and the predictors of obstructive plaques on CTA were determined using univariate- and subsequent multivariate linear regression analyses.
Finally, the predictors of plaque phenotype (number of non-calcified, mixed and calcified plaques) on CTA were evaluated using univariate- and subsequent multivariate linear regression analyses.
Statistical analyses were performed using SPSS software (version 16.0, Inc., Chicago, Illinois). P values <0.05 were considered statistically significant.

Table 1. Characteristics of the patient population (N=103)

Age (years)	54 ± 11
Male sex	53 (51%)
Diabetes mellitus duration (years)	9 ± 7
Smoking	23 (22%)
Family history of CAD	50 (49%)
BMI (kg/m2)	28 (25-34)
Hypertension	69 (67%)
Anti-hypertensive medication use	58 (56%)
Systolic blood pressur (mmHg)	130 (125-145)
Diastolic blood pressure (mmHg)	80 (79-85)
HbA1c (mmol/L)	8.3 (7.3-9.5)
Fasting glucose (mmol/L)	9.6 (7.6-12.6)
Hypercholesterolemia	79 (77%)
Statin use	55 (53%)
Total cholesterol (mmol/L)	4.6 (3.8-5.5)
LDL-cholesterol (mmol/L)	2.9 (2.3-3.6)
HDL-cholesterol (mmol/L)	1.2 (1.0-1.5)
Triglycerides (mmol/L)	1.4 (1.0-2.5)
Micro-albuminuria	31 (30%)

Data are averages ± standard deviation, median (lower quartile – upper quartile) or number of patients (%).CAD: coronary artery disease, BMI: body mass index, HbA1c: glycosylated hemoglobin A1c, LDL: Low-density lipoprotein, HDL: high-density lipoprotein.

RESULTS

Study Population

In total, 103 asymptomatic patients with type 2 diabetes, with a mean age of 54±11 years were included. Fifty-three patients (51%) were men. Further baseline characteristics and traditional cardiovascular risk factors of the patient population are provided in Table 1. Diabetes treatment comprised of only diet in 5 patients (5%), oral agents in 69 patients (67%) and insulin in 65 patients (63%). At the time of referral, 69 patients (67%) had hypertension, of which 58 patients were treated with anti-hypertensive medication. Ace-inhibitors were used in 32 (31%), beta-blockers in 16 (16%), angiotensin-II receptor antagonists in 23 (22%), calcium channel blockers in 10 (10%) and diuretics in 16 patients (16%). Seventy-nine patients (77%) had hypercholesterolemia, and 55 patients (53%) were treated with statins. Furthermore, a minority of 26 patients (26%) received aspirin therapy at referral.

Assessment of coronary atherosclerosis by CTA

As shown in Appendix 1 (page 59), CTA revealed normal coronaries in 36 patients (35%), whereas the remaining 67 patients (65%) were shown to have coronary atherosclerosis. Within this group, 39 patients (38%) had non-obstructive atherosclerosis and 28 patients (27%) had obstructive atherosclerosis.

Overall, the mean number of plaques was 9.3±11.3, and the mean number of obstructive plaques was 1.5±3.2. Analysis of plaque phenotype in the total population showed a predominance of non-calcified plaques (62%), as compared to a minority of mixed (13%) and calcified plaques (25%) (Appendix 1).

Plasma hsCRP and adiponectin levels

Measurement of plasma hsCRP resulted in an overall median value of 2.3 mg/L with an inter-quartile range of 1.1 – 5.8 mg/L.

The overall median plasma adiponectin was determined to be 6.6 mcg/ml (3.8 – 11.2). The term of interaction was not significant between adiponectin and age, duration of diabetes, smoking, family history of CAD, BMI, HbA1c, total cholesterol, LDL-cholesterol or hsCRP levels. A positive significant relation was observed between plasma adiponectin levels and HDL-cholesterol (Spearman's correlation coefficient 0.45, P<0.001). An inverse relation was observed between plasma adiponectin and triglycerides (Spearman's correlation coefficient -0.16, P=0.01) as well as with the male sex (Spearman's correlation coefficient -0.25, P=0.01).

Relation of plasma adiponectin with coronary atherosclerosis on CTA

As illustrated in Appendix 2 (page 60), median plasma adiponectin decreased only modestly from 9.6 mcg/ml (5.4-13.2) in patients with no atherosclerosis to 7.5 mcg/ml (4.7-11.1) in presence of non-obstructive atherosclerosis on CTA (P=0.23). Importantly, a significant further decrease was observed in the median plasma adiponectin of patients with obstructive coronary atherosclerosis (3.8 mcg/ml (2.8-4.7)) (P<0.001) (Appendix 2).

Predictors of the presence of any atherosclerosis and obstructive atherosclerosis

Using univariate logistic regression analysis, age, a positive family history for CAD, hypertension, low HDL-cholesterol, triglycerides, micro-albuminuria and low plasma adiponectin were identified as potential predictors of the presence of any coronary atherosclerosis on CTA (Table 2). Of note, plasma adiponectin maintained a significant inverse relation with the presence of coronary atherosclerosis (P=0.02), after correction for other predictors of any coronary atherosclerosis.

Similarly, age, male sex, low HDL-cholesterol, micro-albuminuria and low plasma adiponectin were shown to have a significant relation with the presence of obstructive

Table 2. Predictors of the presence of any atherosclerosis and obstructive atherosclerosis on CTA.

	Any Atherosclerosis				Obstructive Atherosclerosis			
Variable	*Univariate*		*Multivariate*		*Univariate*		*Multivariate*	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Age	1.10 (1.05-1.16)	**<0.001**	1.12 (1.04-1.19)	**0.001**	1.09 (1.03-1.14)	**0.001**	1.10 (1.04-1.17)	**0.002**
Male sex	2.18 (0.94-5.07)	0.07	-	-	3.33 (1.29-8.60)	**0.013**	1.79 (0.51-6.35)	0.37
Duration of DM	1.03 (0.97-1.10)	0.36	-	-	1.00 (0.94-1.07)	0.99	-	-
Smoker	0.75 (0.28-1.99)	0.56	-	-	1.40 (0.50-3.95)	0.53	-	-
Family history of CAD	3.65 (1.51-8.87)	**0.004**	2.71 (0.82-8.95)	0.10	1.48 (0.61-3.60)	0.39	-	-
Body mass index	1.02 (0.96-1.08)	0.59	-	-	0.97 (0.90-1.08)	0.39	-	-
Hypertension	3.80 (1.57-9.18)	**0.003**	2.09 (0.58-7.46)	0.26	2.28 (0.82-6.35)	0.12	-	-
HbA1c	1.17 (0.92-1.48)	0.20	-	-	1.27 (0.99-1.62)	0.054	-	-
HDL-cholesterol	0.29 (0.10-0.87)	**0.03**	0.78 (0.17-3.59)	0.75	0.23 (0.06-0.94)	**0.04**	1.21 (0.17-8.75)	0.85
Triglycerides	1.68 (1.04-2.71)	**0.03**	1.32 (0.76-2.31)	0.33	1.25 (0.90-1.74)	0.19	-	-
Micro-albuminuria	3.10 (1.13-8.53)	**0.03**	1.73 (0.41-7.28)	0.46	2.79 (1.11-7.02)	**0.03**	3.54 (0.96-13.0)	0.06
Plasma hsCRP	1.08 (0.98-1.19)	0.12	-	-	1.08 (0.99-1.16)	0.054	-	-
Plasma Adiponectin	0.88 (0.81-0.96)	**0.004**	0.85 (0.74-0.97)	**0.02**	0.71 (0.59-0.85)	**<0.001**	0.68 (0.53-0.88)	**0.003**

OR: odds ration, CAD: coronary artery disease, HbA1c: glycosylated hemoglobin A1c, HDL: high-density lipoprotein, hsCRP: high sensitivity C-reactive protein.

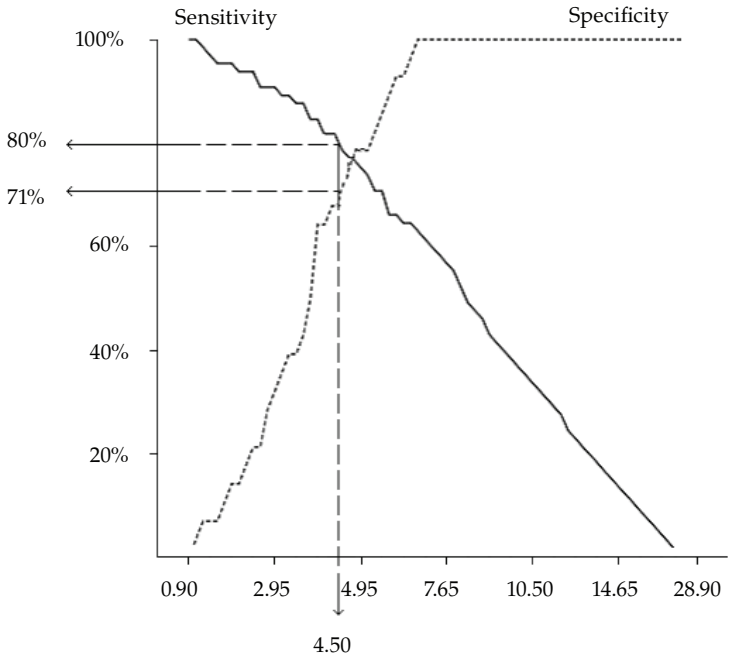


Figure 1. Predictive value of plasma adiponectin for the presence of obstructive coronary atherosclerosis. ROC curve analysis yielded a sensitivity and specificity of 80% and 71% with a plasma adiponectin cut-off value of <4.5 mcg/ml for prediction of obstructive coronary atherosclerosis.

coronary atherosclerosis (Table 2). Importantly, analysis in a multiple logistic regression model, revealed low plasma adiponectin to be an independent predictor of the presence of obstructive coronary atherosclerosis on CTA (P=0.003).

Using ROC curve analysis a cut-off value of 4.50 mcg/ml was identified for plasma adiponectin level. This cut-off value yielded a sensitivity and a specificity of 80% and 71% for predicting obstructive coronary atherosclerosis on CTA, in asymptomatic patients with type 2 diabetes (Figure 1).

Predictors of the extent of coronary atherosclerosis

Age, HbA1c, micro-albuminuria, hsCRP and low plasma adiponectin were significantly related with the extent of coronary atherosclerosis, as represented by the number of atherosclerotic plaques on CTA, in a univariate linear regression model (Table 3). The

Table 3. Predictors of the extent of atherosclerosis (number of atherosclerotic plaques and obstructive atherosclerotic plaques) on CTA.

	Number of Atherosclerotic Plaques				**Number of Obstructive Atherosclerotic Plaques**			
Variable	*Univariate*		*Multivariate*		*Univariate*		*Multivariate*	
	ß (95% CI)	P value	ß (95% CI)	P value	ß (95% CI)	P value	ß (95% CI)	P value
Age	0.32 (0.10-0.55)	**0.005**	0.38 (0.18-0.59)	**<0.001**	0.08 (0.05-0.14)	**0.003**	0.09 (0.04-0.14)	**0.001**
Male Gender	4.38 (-0.25-9.01)	0.063	-	-	1.16 (-0.08-2.40)	0.07	-	-
Duration of DM	0.26 (-0.08-0.59)	0.13	-	-	0.04 (-0.05-0.13)	0.41	-	-
Smoker	2.36 (-3.20-7.91)	0.40	-	-	1.56 (0.07-3.04)	**0.04**	1.06 (-0.39-2.51)	0.15
Family history of CAD	3.40 (-1.27-8.07)	0.15	-	-	0.76 (-0.49-2.01)	0.23	-	-
Body mass index	-0.13 (-0.48-0.21)	0.44	-	-	-0.03 (-0.12-0.07)	0.56	-	-
Hypertension	4.71 (-0.14-9.56)	0.06	-	-	1.29 (-0.02-2.60)	0.053	-	-
HbA1c	1.36 (0.09-2.62)	**0.02**	0.80 (-0.43-2.02)	0.20	0.37 (0.04-0.71)	**0.03**	0.23 (-0.11-0.57)	0.19
HDL-cholesterol	-2.27 (-8.22-3.67)	0.45	-	-	-1.64 (-3.21-(-0.08))	**0.04**	-0.47 (-2.14-1.20)	0.58
Triglycerides	0.18 (-0.47-0.84)	0.58	-	-	0.22 (0.05-0.40)	**0.01**	0.12 (-0.39-0.64)	0.63
Micro-albuminuria	4.97 (0.05-9.90)	**0.048**	1.69 (-2.95-6.33)	0.47	2.23 (0.95-3.51)	**0.001**	1.40 (-0.01-2.82)	0.05
Plasma hsCRP	0.65 (0.24-1.05)	**0.002**	0.64 (0.24-1.03)	**0.002**	0.06 (-0.05-0.17)	0.29	-	-
Plasma Adiponectin	-0.52 (-0.95-0.08)	**0.02**	-0.48 (-0.87-(-0.09))	**0.02**	-0.18 (-0.29-(-0.07))	**0.002**	-0.13 (-0.25-(-0.03)	**0.04**

CAD: coronary artery disease, HbA1c: glycosylated hemoglobin A1c, HDL: high-density lipoprotein, hsCRP: high sensitivity C-reactive protein.

Table 4. Predictors of atherosclerotic plaque phenotype (number of non-calcified, mixed and calcified plaques) on CTA.

	Number of Non-Calcified Plaques				Number of Mixed Plaques				Number of Calcified Plaques			
Variable	*Univariate*		*Multivariate*		*Univariate*		*Multivariate*		*Univariate*		*Multivariate*	
	ß (95% CI)	P value	ß (95% CI)	P value	ß (95% CI)	P value	ß (95% CI)	P value	ß (95% CI)	P value	ß (95% CI)	P value
Age	0.1 (-0.1-0.23)	0.27	-	-	0.1 (0.0-0.1)	**0.03**	0.0 (-0.0-0.1)	0.12	0.2 (0.1-0.3)	**<0.001**	0.1 (0.1-0.2)	**0.002**
Male Gender	1.8 (-1.8-5.3)	0.33	-	-	0.4 (-0.7-1.4)	0.47	-	-	2.2 (0.6-3.8)	**0.01**	0.5 (-0.4-1.4)	0.29
Duration of DM	-0.0 (-0.3-0.2)	0.84	-	-	0.1 (0.1-0.2)	**0.001**	0.1 (0.0-0.1)	0.08	0.2 (0.0-0.3)	**0.013**	0.1 (-0.0-0.2)	0.19
Smoker	1.0 (-3.2-5.3)	0.63	-	-	1.0 (-0.2-2.3)	0.09	-	-	0.3 (-1.8-2.3)	0.79	-	-
Family history of CAD	1.7 (-1.8-5.3)	0.33	-	-	0.4 (-0.6-1.4)	0.45	-	-	1.0 (-0.7-2.7)	0.23	-	-
Body mass index	-0.1 (-0.4-0.2)	0.41	-	-	-0.02 (-0.1-0.1)	0.65	-	-	-0.0 (-0.1-0.1)	0.95	-	-
Hypertension	0.2 (-3.6-3.9)	0.92	-	-	1.5 (0.5-2.5)	**0.004**	0.7 (-0.4-1.8)	0.21	3.0 (1.3-4.6)	**0.001**	0.6 (-0.5-1.7)	0.29
Hb A1c	0.5 (-0.4-1.5)	0.28	-	-	0.26 (0.0-0.5)	0.08	-	-	0.5 (-0.0-0.9)	0.05	-	-
HDL-cholesterol	-1.2 (-5.7-3.3)	0.61	-	-	-0.2 (-1.5-1.1)	0.76	-	-	-0.8 (-2.9-1.3)	0.47	-	-
Triglycerides	-0.1 (-0.6-0.4)	0.80	-	-	0.0 (-0.1-0.2)	0.54	-	-	0.2 (0.0-0.5)	0.07	-	-
Micro-albuminuria	0.8 (-3.0-4.6)	0.69			1.7 (0.7-2.8)	**0.002**	0.7 (-0.4-1.8)	0.19	2.1 (0.4-3.9)	**0.02**	0.9 (-0.2-2.0)	0.10
Plasma hsCRP	0.6 (0.3-0.9)	**<0.001**	0.6 (0.3-0.9)	**0.001**	0.1 (0.01-0.2)	**0.03**	0.1 (0.0-0.2)	0.08	-0.0 (-0.2-0.2)	0.93	-	-
Plasma Adiponectin	-0.4 (-0.7-0)	**0.03**	-0.3 (-0.60-0)	**0.04**	0.0 (-0.1-0.8)	0.79	-	-	-0.1 (-0.3-0.0)	0.12	-	-

CAD: coronary artery disease, HbA1c: glycosylated hemoglobin A1c, HDL: high-density lipoprotein, hsCRP: high sensitivity C-reactive protein.

inverse relation between plasma adiponectin with the extent of coronary atherosclerosis remained significant after correction in a multivariate linear regression model (P=0.02). Likewise, as shown in Table 3, low plasma adiponectin was shown to be independently associated with the number of obstructive coronary atherosclerotic plaques on CTA (P=0.04).

Predictors of atherosclerotic plaque phenotype

The relation of the traditional cardiovascular risk factors, hsCRP and plasma adiponectin with coronary atherosclerotic plaque phenotype is provided in Table 4. Briefly, hsCRP (P=0.001) and low plasma adiponectin (P=0.04) were shown to be independently associated with the number of non-calcified coronary atherosclerotic plaques on CTA. Age, duration of diabetes, hypertension, micro-albuminuria and hsCRP were associated with mixed atherosclerotic plaques, but all lost significance in a multi-variate linear regression model. Age was shown to be the only independent factor associated with the number of calcified coronary atherosclerotic plaques on CTA (P=0.002).

In consequence, plasma adiponectin was independently associated with the quantity of non-calcified, but not with mixed or calcified coronary atherosclerotic plaques (Table 4).

DISCUSSION

In the current study of asymptomatic patients with type 2 diabetes, an inverse relation was observed between plasma adiponectin and the presence and extent of coronary atherosclerosis. Of note, the relation between low adiponectin and coronary atherosclerosis remained significant after correction for traditional cardiovascular risk factors and hsCRP. Low adiponectin was strongly related with obstructive atherosclerosis. Accordingly, an adiponectin cut-off value of <4.5 mcg/ml resulted in a sensitivity of 80% for predicting obstructive atherosclerosis in asymptomatic patients with type 2 diabetes. Whereas no relation was observed between adiponectin and mixed or calcified coronary atherosclerotic plaques, an inverse association was shown between adiponectin and the quantity of non-calcified plaques. Thereby, in asymptomatic patients with type 2 diabetes, low plasma adiponectin predominantly contributed to the presence and extent of coronary atherosclerosis by predisposing non-calcified coronary atherosclerotic plaques.

Assessment of coronary atherosclerosis by CTA

CTA provides imaging of the structure and composition of the coronary arteries, thus allowing the evaluation of atherosclerosis. Importantly, in diabetic patients the diagnostic accuracy of CTA for the detection of obstructive atherosclerosis has been shown to be excellent, with a sensitivity of approximately 95% [20]. In addition, the technique

provides information on atherosclerotic plaque burden and plaque composition [19]. In retrospective studies, calcified coronary plaques have been associated with advanced but stable stages of atherosclerosis [21]. On the contrary, non-calcified plaques have been linked to the relatively early but more unstable stages of the disease [21]. Thus far limited studies have addressed the risk factors associated with the presence of coronary atherosclerosis and its morphology in asymptomatic patients with type 2 diabetes [22]. Due to its non-invasive nature, multi-slice CTA of the coronaries provides the possibility to assess atherosclerosis in asymptomatic patients with type 2 diabetes. Thereby, coronary atherosclerosis and its morphology can be evaluated in relation to both traditional risk factors and novel biomarkers, thus improving the understanding of pathophysiology of atherosclerosis in type 2 diabetes, as well as enabling targeted management strategies.

Relation of adiponectin with coronary atherosclerosis

Based on in vitro and animal studies, low adiponectin has been suggested to be an important causal link between adipose tissue dysfunction and atherosclerosis [23-27]. Adiponectin inhibits atherosclerosis by acting as an endogenous modulator of endothelial function through suppressing adhesion molecules [23], by inhibiting NF-κB [24], an interaction with interleukin-10 against vascular inflammation [25] and reduction of cholesterol uptake in macrophages and their transformation into foam cells [26]. In addition, adiponectin reduces vascular smooth muscle cell proliferation, migration and apoptosis [27]. A number of genetic studies confirm and extent the evidence implicating anti-atherogenic effects of adiponectin [8,28,29]. For instance, administration of recombinant adenovirus expressing human adiponectin to apoE-deficient animals caused a 30% reduction in the formation of atherosclerotic plaques [28]. Furthermore, in a meta-analysis of 827 individuals with CAD and 1887 without CAD, the adiponectin gene variant with the polymorphism +276GàT was associated with a 45% decreased risk for CAD [29].

Nevertheless, the predictive value of plasma adiponectin for cardiovascular events has been variable in the general population [9-11]. In an initial 6 years follow-up study of 18225 male participants, individuals with the highest quintile of adiponectin (24.9-56.1 mcg/mL) compared with the lowest (2.4-10.5 mcg/mL), showed a substantial decrease in risk of coronary events even after correction for other cardiovascular risk factors (RR 0.56, 95% CI 0.32-0.99) [9]. In contrast, in a prospective study and meta-analysis by Sattar and colleagues, comparison of men in the top adiponectin tertile with the lowest tertile, revealed a much more moderate association with coronary events (OR 0.84, 95% CI 0.70-1.01) [11]. However, in cross-sectional studies, low thresholds of plasma adiponectin of <4.0-5.5 mg/L have been consistently associated with CAD [12,13].

Adiponectin as a predictor of coronary atherosclerosis in type 2 diabetes

In the general population, a large variation is observed in the plasma adiponectin levels, which range up to 25 mcg/mL [11]. As a consequence, adiponectin levels may be a poor marker of coronary atherosclerosis in the general population. On the other hand, coherent to adipose tissue dysfunction, adiponectin levels are relatively low in patients with type 2 diabetes and mainly range 2-12 mcg/mL [14]. Therefore, assessment of adiponectin particularly in patients with type 2 diabetes is likely to yield a higher rate of low adiponectin levels, which predispose coronary atherosclerosis. Consequently, adiponectin may be a more effective marker of coronary atherosclerosis in type 2 diabetes.

As in the general population, the pathogenesis of atherosclerosis is multi-factorial in type 2 diabetes. However, excessive visceral adipose tissue which prompts insulin resistance, may play a more pivotal role in clustering of cardiovascular risk and development of atherosclerosis in patients with type 2 diabetes [6]. As visceral adipose tissue expands, macrophages infiltrate adipose tissue resulting in adipose tissue dysfunction [30]. This phenomenon increases the production of adipocytokines involved in glucose metabolism (e.g. resistin), lipid metabolism (e.g. cholesterol ester transfer protein), coagulation (plasminogen activator inhibitor-1) and inflammation (e.g. tumor necrosis factor-α, interleukin-6, C-reactive protein) [6]. Only the production of the adipocytokine adiponectin decreases during this process [6]. Being produced by adipocytes, a low plasma adiponectin concentration is a good marker of adipose tissue dysfunction [31].

The results of the current study indeed confirm an association between adiponectin and the presence, degree and extent of coronary atherosclerosis in asymptomatic patients with type 2 diabetes. Of note, a plasma adiponectin of <4.5 mcg/mL was associated with a sensitivity of 80% for the presence of obstructive atherosclerosis in this population of patients. These findings confirm previous observations in patients with type 2, wherein adiponectin was found to be inversely associated with coronary events [15, 16]. In a study by Schulze et al, the predictive value of adiponectin was attenuated by HDL cholesterol [15]. In contrast, in the current population of asymptomatic patients with type 2 diabetes, low adiponectin was shown to be associated with the presence, degree and extent of atherosclerosis also after correction for traditional risk factors (including low HDL cholesterol) and hsCRP.

In line with the current study results, previous studies in asymptomatic patients with type 2 diabetes have revealed a high prevalence of obstructive atherosclerosis (26-34%) and a high proportion of non-calcified coronary plaques (41-66%) [32,33]. Prognostic data suggest the presence of not only obstructive atherosclerotic plaques, but also non-calcified coronary plaques to convey an increased risk for coronary events [34].

Importantly, low adiponectin, akin to hsCRP (a marker of inflammation), was shown to be especially related with non-calcified atherosclerotic plaques.
Use of a biomarker, such as adiponectin, for the cardiovascular risk stratification of asymptomatic patients with type 2 diabetes may comprise several advantages. Potentially, patients at risk for CAD, could be distinguished at an early stage, prior to clinically manifest atherosclerosis and treated more aggressively. In addition, application of a biomarker may provide a more general estimation of atherosclerotic risk, opposing non-invasive vascular or cardiac imaging techniques which are often restricted to a certain aspect of vascular disease or a specific organ. On the other hand, clinical utility of biomarkers has often been limited by their low specificity [35]. Although several biomarkers (e.g. hsCRP) show a strong correlation with CAD in study populations, the inverse claim that elevated markers indicate a high risk has often been difficult to substantiate [35]. Remarkably however, in this study of asymptomatic patients with type 2 diabetes, low adiponectin (<4.5 mcg/mL), was not only associated with a good sensitivity, but also with a reasonable specificity of 71% for predicting obstructive coronary atherosclerosis.

Study limitations

The current study was limited to the evaluation of the relation between adiponectin and coronary atherosclerosis in patients with type 2 diabetes, and assessment did not include a non-diabetic control group. As CTA is accompanied with radiation exposure, it is not feasible to perform a similar assessment in asymptomatic subjects free of cardiovascular risk. Furthermore, the prognostic value of adiponectin was not evaluated as no follow-up data were available.

CONCLUSION

Low plasma adiponectin was independently related with the presence, degree and extent of coronary atherosclerosis in asymptomatic patients with type 2 diabetes. A predominant association was observed with the presence of non-calcified plaques. Assessment of plasma adiponectin conveys the potential to augment risk for CAD in asymptomatic patients with type 2 diabetes.

REFERENCES

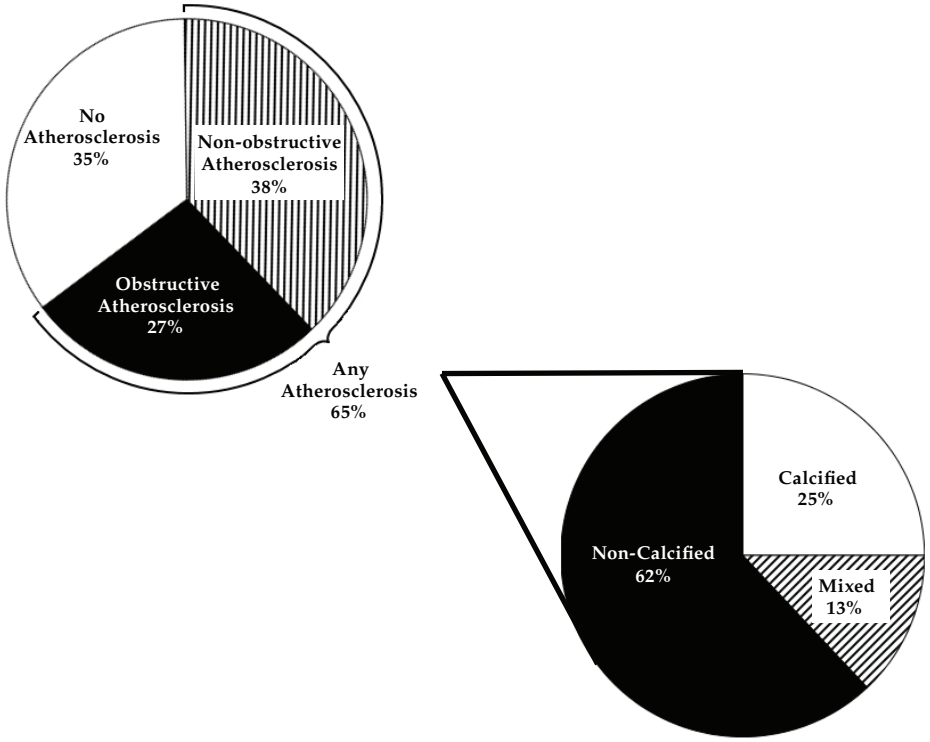
1. Gregg EW, Gu Q, Cheng YJ, Narayan KM, Cowie CC. Mortality trends in men and women with diabetes, 1971 to 2000. Ann Intern Med 2007;147:149-155.
2. Wackers FJ, Young LH, Inzucchi SE, Chyun DA, Davey JA, Barrett EJ, Taillefer R, Wittlin SD, Heller GV, Filipchuk N, Engel S, Ratner RE, Iskandrian AE. Detection of Ischemia in Asymptomatic Diabetics Investigators. Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. Diabetes Care 2004;27: 1954-1961.
3. Coleman RL, Stevens RJ, Retnakaran R, Holman RR. Framingham, SCORE, and DECODE risk equations do not provide reliable cardiovascular risk estimates in type 2 diabetes. Diabetes Care 2007;30:1292-1293b.
4. Raggi P. Role of electron-beam computed tomography and nuclear stress testing in cardiovascular risk assessment. Am J Cardiol 2005;96:20J-27J.
5. St Clair L, Ballantyne CM. Biological surrogates for enhancing cardiovascular risk prediction in type 2 diabetes mellitus. Am J Cardiol 2007;99:80B-88B.
6. Hajer GR, van Haeften TW, Visseren FL. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. Eur Heart J 2008;29:2959-2971.
7. Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, Knowler WC, Krakoff J. Adiponectin and development of type 2 diabetes in the Pima Indian population. Lancet 2002;360:57-58.
8. Menzaghi C, Trischitta V, Doria A. Genetic influences of adiponectin on insulin resistance, type 2 diabetes, and cardiovascular disease. Diabetes 2007;56:1198-1209.
9. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA 2004;291:1730-1737.
10. Ai M, Otokozawa S, Asztalos BF, White CC, Cupples LA, Nakajima K, Lamon-Fava S, Wilson PW, Matsuzawa Y, Schaefer EJ. Adiponectin: an independent risk factor for coronary heart disease in men in the Framingham offspring Study. Atherosclerosis 2011;217:543-548.
11. Sattar N, Wannamethee G, Sarwar N, Tchernova J, Cherry L, Wallace AM, Danesh J, Whincup PH. Adiponectin and coronary heart disease: a prospective study and meta-analysis. Circulation 2006;114:623-629.
12. Wolk R, Berger P, Lennon RJ, Brilakis ES, Davison DE, Somers VK. Association between plasma adiponectin levels and unstable coronary syndromes. Eur Heart J 2007;28:292-298.
13. Nakamura Y, Shimada K, Fukuda D, Shimada Y, Ehara S, Hirose M, Kataoka T, Kamimori K, Shimodozono S, Kobayashi Y, Yoshiyama M, Takeuchi K, Yoshikawa J. Implications of plasma concentrations of adiponectin in patients with coronary artery disease. Heart 2004;90:528-533.
14. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y. Plasma concentrations

of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000; 20:1595-1599.

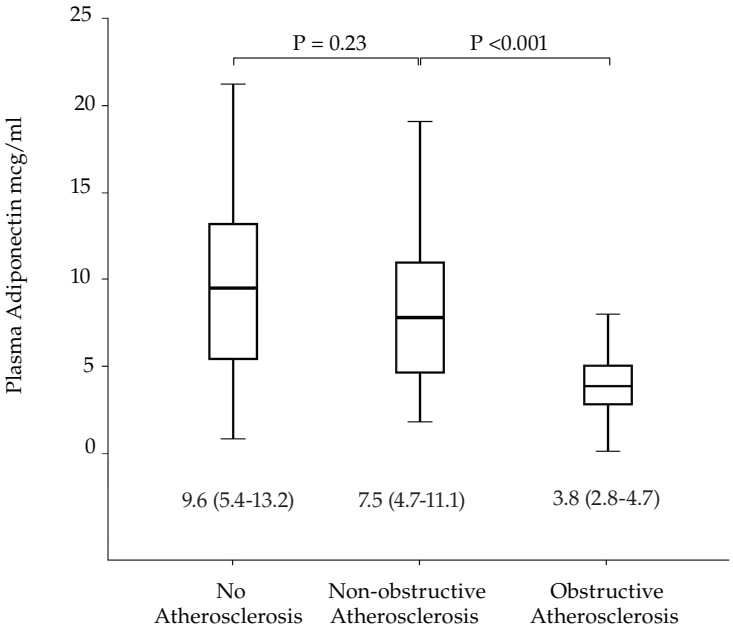
15. Schulze MB, Shai I, Rimm EB, Li T, Rifai N, Hu FB. Adiponectin and future coronary heart disease events among men with type 2 diabetes. Diabetes 2005;54:534-539.
16. Lim S, Koo BK, Cho SW, Kihara S, Funahashi T, Cho YM, Kim SY, Lee HK, Shimomura I, Park KS. Association of adiponectin and resistin with cardiovascular events in Korean patients with type 2 diabetes: the Korean atherosclerosis study (KAS): a 42-month prospective study. Atherosclerosis 2008;196:398-404.
17. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183.
18. Rose G, McCartney P, Reid DD. Self-administration of a questionnaire on chest pain and intermittent claudication. Br J Prev Soc Med 1977;31:42-48.
19. Leber AW, Knez A, Becker A, Becker C, von Ziegler F, Nikolaou K, Rist C, Reiser M, White C, Steinbeck G, Boekstegers P. Accuracy of multidetector spiral computed tomography in identifying and differentiating the composition of coronary atherosclerotic plaques: a comparative study with intracoronary ultrasound. J Am Coll Cardiol 2004;43:1241-1247.
20. Schuijf JD, Bax JJ, Jukema JW, Lamb HJ, Vliegen HW, Salm LP, de Roos A, van der Wall EE. Noninvasive angiography and assessment of left ventricular function using multislice computed tomography in patients with type 2 diabetes. Diabetes Care 2004;27:2905-2910.
21. Hoffmann U, Moselewski F, Nieman K, Jang IK, Ferencik M, Rahman AM, Cury RC, Abbara S, Joneidi-Jafari H, Achenbach S, Brady TJ. Noninvasive assessment of plaque morphology and composition in culprit and stable lesions in acute coronary syndrome and stable lesions in stable angina by multidetector computed tomography. J Am Coll Cardiol 2006;47:1655-1662.
22. Bernard S, Loffroy R, Sérusclat A, Boussel L, Bonnefoy E, Thévenon C, Rabilloud M, Revel D, Moulin P, Douek P. Increased levels of endothelial microparticles CD144 (VE-Cadherin) positives in type 2 diabetic patients with coronary noncalcified plaques evaluated by multidetector computed tomography (MDCT). Atherosclerosis 2009;203:429-435.
23. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 1999;100:2473-2476.
24. Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, Hotta K, Nishida M, Takahashi M, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation 2000;102:1296-1301.

25. Kumada M, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K, Maeda K, Nagaretani H, Kishida K, Maeda N, Nagasawa A, Funahashi T, Matsuzawa Y. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. Circulation 2004;109:2046-2049.
26. Hopkins TA, Ouchi N, Shibata R, Walsh K. Adiponectin actions in the cardiovascular system. Cardiovasc Res 2007;74:11-18.
27. Goldstein BJ, Scalia R. Adiponectin: A novel adipokine linking adipocytes and vascular function. J Clin Endocrinol Metab 2004;89:2563-2568.
28. Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, Ohashi K, Sakai N, Shimomura I, Kobayashi H, Terasaka N, Inaba T, Funahashi T, Matsuzawa Y. Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. Circulation 2002;106:2767-2770.
29. Qi L, Doria A, Manson JE, Meigs JB, Hunter D, Mantzoros CS, Hu FB. Adiponectin genetic variability, plasma adiponectin, and cardiovascular risk in patients with type 2 diabetes. Diabetes 2006;55:1512-1516.
30. Cancello R, Tordjman J, Poitou C, Guilhem G, Bouillot JL, Hugol D, Coussieu C, Basdevant A, Bar Hen A, Bedossa P, Guerre-Millo M, Clément K. Increased infiltration of macrophages in omental adipose tissue is associated with marked hepatic lesions in morbid human obesity. Diabetes 2006;55:1554-1561.
31. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 1995;270:26746-26749.
32. Scholte AJ, Schuijf JD, Kharagjitsingh AV, Jukema JW, Pundziute G, van der Wall EE, Bax JJ. Prevalence of coronary artery disease and plaque morphology assessed by multi-slice computed tomography coronary angiography and calcium scoring in asymptomatic patients with type 2 diabetes. Heart 2008;94:290-295.
33. Djaberi R, Schuijf JD, Boersma E, Kroft LJ, Pereira AM, Romijn JA, Scholte AJ, Jukema JW, Bax JJ. Differences in atherosclerotic plaque burden and morphology between type 1 and 2 diabetes as assessed by multislice computed tomography. Diabetes Care 2009;32:1507-1512.
34. van Werkhoven JM, Schuijf JD, Gaemperli O, Jukema JW, Boersma E, Wijns W, Stolzmann P, Alkadhi H, Valenta I, Stokkel MPM, Kroft LJ, de Roos A, Pundziute G, Scholte A, van der Wall EE, Kaufmann P, Bax JJ. Prognostic value of multi-slice computed tomography and gated single photon emission computed tomography in patients with suspected coronary artery disease. J Am Coll Cardiol 2009;53:623-632.
35. Eijgelaar WJ, Heeneman S, Daemen MJ. The vulnerable patient: refocusing on the plaque? Thromb Haemost 2009;102:231-239.

SUPPLEMENTARY FIGURES



Appendix 1. Results of CTA in asymptomatic patients with type 2 diabetes. Pie-chart on the top left presents the results of CTA at patient level. Pie-chart on the bottom right presents the distribution of plaque phenotype in patients with atherosclerotic plaques.



Appendix 2. Relation of plasma adiponectin with coronary atherosclerosis as assessed by CTA. Plasma adiponectin decreased with the presence and degree of coronary atherosclerosis.

CHAPTER 4

Differences in Atherosclerotic Plaque Burden and Morphology between Type 1 and 2 Diabetes Mellitus as Assessed by Multi-Slice Computed Tomography

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Diabetes Care. 2009;32:1507-1512

ABSTRACT

Objective

It is unclear whether coronary atherosclerotic plaque burden is similar in patients with type 1 and type 2 diabetes. By using multi-slice CT (MSCT) the presence, degree and morphology of coronary artery disease (CAD) in type 1 and type 2 diabetes were compared.

Research design and methods

Prospectively, coronary artery calcium (CAC) scoring and MSCT coronary angiography were performed in 135 asymptomatic patients (65 patients with type 1 and 70 patients with type 2 diabetes). The presence and extent of coronary atherosclerosis as well as plaque phenotype were assessed and compared between groups.

Results

No difference was observed in average CAC score (217±530 vs. 174±361) nor the prevalence of coronary atherosclerosis (65% vs. 71%) in type 1 and type 2 diabetes. However, the prevalence of obstructive atherosclerosis was higher in patients with type 2 diabetes (n=24; 34%) as compared to type 1 diabetes (n=11; 17%) (P=0.02). Also, higher mean number of atherosclerotic and obstructive plaques was observed in type 2 diabetes. In addition, the percentage of non-calcified plaques was higher in type 2 diabetes (66%) versus type 1 diabetes (27%) (P<0.001), resulting in a higher plaque burden for each CAC score as compared to type 1 diabetic patients.

Conclusions

Although CAC scores and prevalence of coronary atherosclerosis were similar between patients with type 1 and type 2 diabetes, CAD was more extensive in the latter. Also, a relatively higher proportion of non-calcified plaques was observed in type 2 diabetes. These observations may be valuable in the development of targeted management strategies adapted to diabetes type.

INTRODUCTION

Cardiovascular disease and coronary artery disease (CAD) in particular constitute a major cause of morbidity and mortality in patients with diabetes (1). However, management of this patient population remains challenging. Current European guidelines regard type 2 diabetes as a CAD equivalent, whereas type 1 diabetes is considered a high risk state only in presence of microalbuminuria (2). In contrast, US guidelines on primary prevention recommend stringent pharmacological therapy with lipid and blood pressure goals comparable to those in secondary prevention in all diabetic patients regardless of type (3). Notably, these guidelines are based on clinical trials in type 2 diabetes. However, CAD in type 1 diabetes, which has been studied less extensively, may have a distinct pathophysiology from type 2 diabetes. As a result, caution is indicated when extrapolating clinical observations obtained in type 2 diabetes to patients with type 1 diabetes. To date, it is unclear whether asymptomatic type 1 diabetic patients equally benefit from the current preventive treatment strategies.

To optimize guidelines for type 1 diabetic patients, more detailed understanding of coronary atherosclerosis in type 1 diabetes is required. Thus far, most studies have evaluated the complications and risk factors associated with microvascular disease in this population (4). Studies of CAD have focused mainly on type 1 diabetic patients with kidney failure undergoing coronary angiography before kidney transplantation (5). Limited information is available on the presence and morphology of CAD in asymptomatic patients with type 1 diabetes in daily clinical practice (6).

Multi-slice Computed Tomography (MSCT) allows evaluation of coronary artery calcium (CAC) score and direct assessment of coronary artery integrity. Importantly, in diabetic patients the diagnostic accuracy of MSCT coronary angiography for the detection of significant stenoses has been shown to be similar to the general population (7). In addition, the technique provides information on atherosclerotic plaque burden and to some extent on plaque composition (8-10). Previous studies with MSCT revealed an increased prevalence of non-calcified coronary plaques, which have been linked to unstable CAD, in type 2 diabetes (11,12). However, thus far no studies have addressed plaque morphology in patients with type 1 diabetes. To improve understanding of potential differences in pathophysiology and atherosclerotic patterns as well as for development of more targeted management strategies, the evaluation of differences in plaque composition on MSCT may provide valuable information.

Therefore, the purpose of the present study was to explore and compare the extent, degree and morphology of coronary atherosclerosis by MSCT in asymptomatic patients with type 1 and type 2 diabetes recruited from a regular diabetes clinic.

RESEARCH DESIGN AND METHODS

Patients and design

A total of 135 consecutive asymptomatic patients with DM were prospectively included from an ongoing registry of new patients at the diabetes outpatient clinic. Diabetic patients were referred to the cardiology outpatient clinic for cardiovascular screening. Patients were stratified as having type 1 or type 2 diabetes according to the ADA criteria (13). Plasma levels of C-peptide and auto-antibodies to islet cells, insulin and glutamic-acid-decarboxylase (GAD) were determined to distinguish between primary insulinopenia and immune destruction of beta pancreas cells (type 1 diabetes) and insulin resistance (type 2 diabetes).

Asymptomatic status was confirmed using the Rose questionnaire for angina (14). A structured interview, physical examination and laboratory analysis were acquired in all patients. Cardiovascular risk factors were assessed according to the following criteria: *1*) positive family history of CAD (CAD in first degree family members <55 (men) or <65 (women) years of age), *2*) smoking (current smoking or smoking in the last 2 years), *3*) hypertension (blood pressure >140/90 mmHg or treatment with antihypertensive medication), *4*) hypercholesterolemia (total cholesterol level >5.0 mmol/L or use of cholesterol lowering medication), *5*) obesity (estimated by body mass index [BMI=Kg/m^2]), *6*) level of glycemic control defined by plasma HbA1c (mmol/L), *7*) diabetic nephropathy (urine albumin/creatinine ratio ≥35 mg/mmol) and *8*) renal function (estimated by glomerular filtration rate [GFR]).

Non-invasive MSCT coronary angiography was performed in all patients as part of a clinical protocol. Exclusion criteria consisted of ventricular and supraventricular arrhythmia and contraindications for the use of iodinated contrast media.

MSCT data acquisition

Imaging was performed with a 64-slice MSCT scanner (Toshiba Medical Systems, Tokyo, Japan). In case of a heart rate ≥ 65 beats per minute, oral beta-blocking medication (metoprolol 50 mg or 100 mg) was provided 1 hour preceding the scan, if tolerated. First, a prospective CAC scan without contrast enhancement was performed, followed by MSCT coronary angiography according to protocols described previously (15).

Assessment of CAD by MSCT

CAC score

CAC score was assessed using dedicated software (Vitrea2; Vital Images, Minnetonka, MN). CAC was identified as a dense area in the coronary artery exceeding the threshold of 130 Hounsfield units. A total Agatston score was determined for each patient.

Coronary atherosclerosis

All MSCT coronary angiograms were interpreted by two experienced observers blinded to the patients characteristics. Discrepancies in interpretation were immediately resolved by consensus. The presence of coronary atherosclerosis was evaluated by scrolling through axial images, followed by visual assessment of curved multiplanar reconstructions in at least two orthogonal planes. Coronary plaques were defined as structures >1 mm^2 within and/or adjacent to the coronary artery lumen, which could be clearly distinguished from the vessel lumen and the surrounding pericardial tissue (10). First, the presence of any atherosclerosis (≥1 plaque in the coronary tree), multi-vessel atherosclerosis (≥1 plaque in minimum two coronary arteries), and that of obstructive atherosclerosis (luminal narrowing ≥ 50%) were evaluated at the patient level, in type 1 and type 2 diabetic patients. Thereafter, a more extensive analysis of plaque burden was obtained by registering the total number of atherosclerotic plaques and obstructive plaques for each patient (10).

In addition, plaques were classified according to phenotype: *1*) non-calcified plaques (plaques with lower density than contrast-enhanced lumen), *2*) calcified plaques (plaques with higher density than contrast-enhanced lumen), and *3*) mixed plaques (plaques with components of low- and high density plaques) (8-10).

Statistical Analysis

Continuous variables were expressed as means ± standard deviation and compared between the group of patients with type 1 and type 2 diabetes by using the two-tailed independent *t*-test. Categorical variables were expressed as numbers (percentages) or medians (lower-quartile, upper-quartile) and compared with a χ^2-test.

Separate multivariate regression analyses with backward elimination were performed correcting for all baseline clinical characteristics including age, male gender, BMI, smoking, positive family history of CAD, hypercholesterolemia, hypertension, HbA1c, GFR and type of diabetes, to identify independent predictors of each coronary atherosclerosis variable on MSCT.

The relationship between CAC scores and extent of coronary atherosclerosis was compared in type 1 and type 2 diabetes. Patients were further classified according to CAC scores: *1*) patients without coronary calcium, *2*) patients with a CAC score in the range 1-100 and *3*) patients with a CAC score >100. The mean number of atherosclerotic lesions was determined for each CAC score category.

Statistical analyses were performed using SPSS software (version 12.0.1, SPSS, Chicago, Illinois) and SAS software (version 6.12; SAS Institute, Cary, North Carolina). $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

A total of 135 asymptomatic diabetic patients were included in the study. Mean age was 48±10 years and 79 patients (59%) were male. The study population consisted of 65 patients with type 1 and 70 patients with type 2 diabetes. Baseline characteristics are provided in Table 1. Importantly, age and gender distribution were comparable in patients with type 1 and type 2 diabetes. However, patients with type 2 diabetes had a significantly higher BMI and HbA1c and a shorter duration of diagnosed diabetes. Other baseline cardiovascular risk factors were similar in the two groups.

Assessment of CAD by MSCT

CAD at patient level

Results of CAD assessment by MSCT are illustrated in Table 2. The mean CAC score and prevalence of atherosclerosis were similar among patients with type 1 and type 2 diabetes. However, the prevalence of multi-vessel atherosclerosis was higher in patients with type 2 (n = 41; 59%) diabetes than in type 1 (n = 19; 29%) diabetes (P = 0.001). Finally, obstructive stenosis was more prevalent in type 2 diabetes (n = 24; 34%) than in type 1 diabetes (n = 11; 17%) (P = 0.02).

Plaque analysis

Quantification of the total number of atherosclerotic plaques for each patient revealed a significantly higher mean number of lesions in patients with type 2 diabetes (9.9 ± 11.9) as compared to patients with type 1 diabetes (3.4 ± 4.8) (P < 0.001) (Fig. 1*A*). In addition, the mean number of obstructive plaques was significantly higher in type 2 diabetes (1.7 ± 3.9) than in type 1 diabetes (0.5 ± 1.4) (P = 0.02) (Fig. 1*A*).

Analysis of plaque phenotype showed a higher mean number of non-calcified plaques in type 2 diabetes (6.5 ± 9.5) versus type 1 diabetes (1.0 ± 1.3) (P < 0.001), whereas the mean number of mixed- and calcified plaques was not significantly different. Accordingly, a higher proportion of non-calcified plaques was observed in type 2 diabetes (66%) in comparison with type 1 diabetes (27%) (P < 0.001) (Fig. 1*B*).

Relation between type of diabetes and the presence and extent of CAD

To correct for baseline characteristics, the relation between type 2 diabetes (as compared to type 1 diabetes) and the presence and extent of CAD was evaluated using multivariate regression analyses (Table 3). Although type of diabetes was not related to the presence of

Table 1. Characteristics of the study population (n=135)

Characteristic	DM1 (n=65)	DM2 (n=70)	P value
Age (years)	46.0 (38.0-54.5)	49.5 (45.0-57.0)	0.08
Men	42 (65%)	37 (53%)	0.17
BMI (Kg/m^2)	23.8 (22.2-26.6)	28.2 (24.9-33.4)	<0.001
Smokers	17 (26%)	14 (20%)	0.40
Family history of CAD	30 (46%)	37 (53%)	0.44
Hypercholesterolemia	41 (63%)	50 (71%)	0.30
Hypertension	32 (49%)	43 (61%)	0.22
Duration of DM (years)	23.0 (9.5-33.0)	7.5 (2.0-13.0)	<0.001
HbA1c (mmol/L)	7.6 (6.6-8.6)	8.3 (7.0-9.5)	0.04
GFR (mL/min/1.73m^2)	101.6 (84.7-122.8)	98.4 (81.8-124.8)	0.88
Albuminuria*	1 (2%)	4 (6%)	0.20

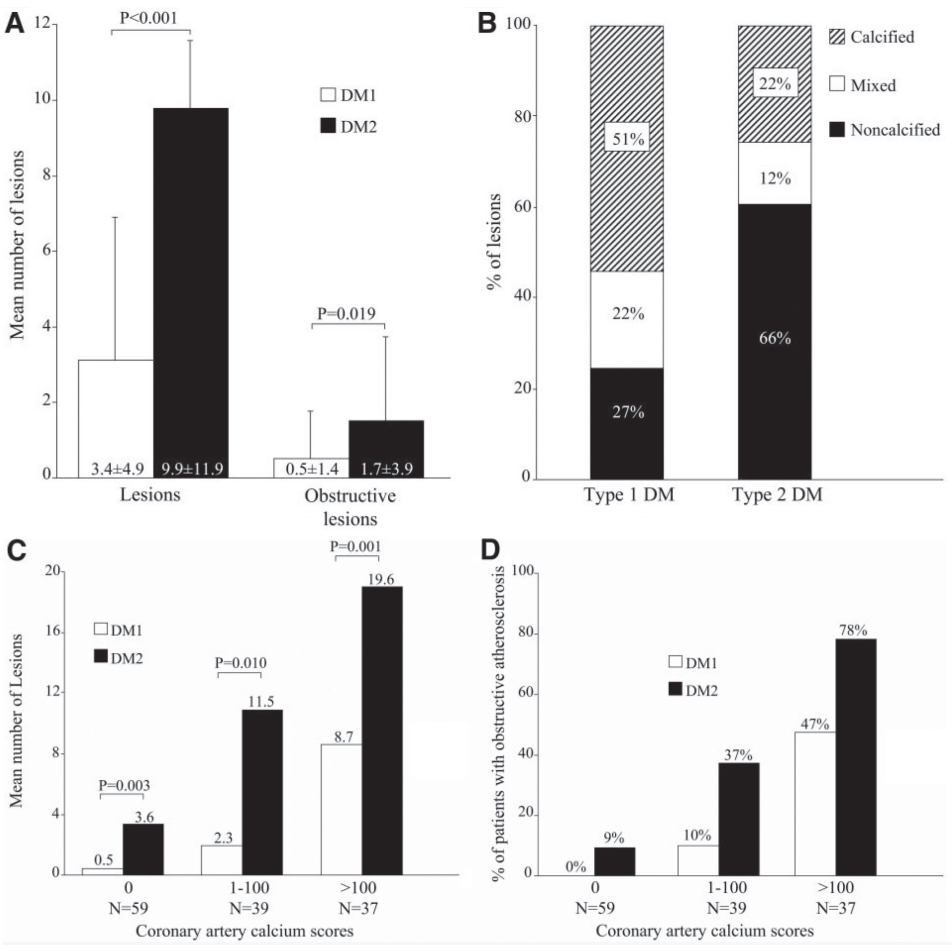
Data are medians (lower quartile, upper quartile) or number of patients (%). BMI = body mass index, CAD = coronary artery disease, DM = diabetes mellitus. *Albuminuria was defined by a urine albumin/creatinine ratio ≥35 mg/mmol.

Table 2. Results of MSCT coronary angiography

		DM1	DM2	P value
Patients				
	Coronary artery calcium scores	217 ± 530	174 ± 361	0.59
	Atherosclerosis	42 (65%)	50 (71%)	0.40
	Atherosclerosis Multi-vessel	19 (29%)	41 (59%)	0.001
	Obstructive Atherosclerosis*	11 (17%)	2 (34%)	0.02
Plaques				
	No of plaques	3.4 ± 4.8	9.9 ± 11.9	<0.001
	No of obstructive plaques	0.5 ± 1.4	1.7 ± 3.9	0.02
	No of non-calcified plaques	1.0 ± 1.3	6.5 ± 9.5	<0.001
	No of mixed plaques	0.7 ± 1.3	1.1 ± 1.9	0.25
	No of calcified plaques	1.8 ± 3.6	2.2 ± 3.9	0.52

Data are mean ± standard deviation, median (lower quartile, upper quartile) or number of patients (%).
* Obstructive Atherosclerosis defined as luminal narrowing ≥50%.

any atherosclerosis, type 2 diabetes strongly related to the extent and degree of coronary atherosclerosis after correction for all other risk factors. In addition, an independent association was observed between the presence of type 2 diabetes and increased number of non-calcified coronary plaques.



Figure

1A. Clustered columns demonstrating average number of lesions and obstructive lesions in type 1 (DM1) and type 2 (DM2) diabetes. A significantly higher mean number of lesions and obstructive lesions were observed in type 2 diabetes.

1B. Bar graph illustrating plaque phenotype in type 1 and type 2 diabetes. A higher percentage of non-calcified plaques was observed in type 2 diabetes.

1C. Clustered bar graph illustrating the increase in number of lesions for each CAC score category among patients with type 1 and type 2 diabetes. Plaque burden was significantly higher in type 2 diabetes for each CAC score category.

1D. Clustered bar graph demonstrating the increase in prevalence of obstructive atherosclerosis for each CAC score category among patients with type 1 and type 2 diabetes. Absence of coronary calcium excluded obstructive atherosclerosis in type 1 diabetes, but not in type 2 diabetes. Prevalence of obstructive atherosclerosis was higher in type 2 diabetes for each CAC score category.

Table 3. Presence of type 2 diabetes (not type 1 diabetes) as a predictor of MSCT variables. Results of multivariate analysis in a backward regression model. Predictive value of type 2 diabetes was tested in a separate multi-variate regression model for each MSCT variable.

		HR or ß (95% CI)	P value
Patients			
	Coronary artery calcium score*	-	NS
	Atherosclerosis†	-	NS
	Atherosclerosis Multi-vessel†	4.16 (1.76-9.93)	0.001
	Obstructive Atherosclerosis‡,†	4.01 (1.38-11.60)	0.01
Plaques			
	No of plaques*	6.82 (3.51-10.13)	<0.001
	No of obstructive plaques*	1.40 (1.32-2.48)	0.01
	No of non-calcified plaques*	6.27 (3.60-8.94)	<0.001
	No of mixed plaques*	-	NS
	No of calcified plaques*	-	NS

NS = Not significant.

* Results of analysis in a multivariate linear regression model.

† Results of analysis in a multivariate binary logistic regression model.

‡ Obstructive Atherosclerosis defined as luminal narrowing ≥50%.

Relation between CAC scores and atherosclerosis

Comparison of the CAC score versus coronary angiography showed that the number of atherosclerotic lesions paralleled the increase in coronary calcium (Fig. 1*C*). However, for each CAC score category the mean number of atherosclerotic lesions was significantly higher in patients with type 2 diabetes (Fig. 1*C*).

Similarly, the prevalence of obstructive atherosclerosis increased per CAC score category (Fig. 1*D*). Importantly, absence of coronary calcium excluded the presence of obstructive atherosclerosis in type 1 diabetes, whereas obstructive atherosclerosis was identified in 3 patients with type 2 diabetes (9%). In patients with a CAC score ≤100, obstructive atherosclerosis was found in only 2 (4%) in type 1 diabetic patients, whereas 10 (19%) type 2 diabetes patients showed obstructive CAD.

CONCLUSIONS

In the present study, no significant difference was observed in the prevalence of atherosclerosis in asymptomatic patients with type 1 and type 2 diabetes. However, in type 1 diabetes, multi-vessel disease was less prevalent and a lower atherosclerotic plaque burden was observed. Moreover, the number of obstructive coronary lesions was

significantly lower in patients with type 1 diabetes. The presence of type 2 diabetes (as opposite to type 1 diabetes) was shown to be an independent predictor of extent and degree of coronary atherosclerosis on MSCT angiography. Second, for each CAC score category a higher atherosclerotic plaque burden was observed in type 2 diabetes. This observation was explained by the high proportion of non-calcified plaques in asymptomatic patients with type 2 diabetes compared with a high proportion of calcified plaques in type 1 diabetes.

Plaque burden

Most previous studies on the prevalence of coronary atherosclerosis have used conventional coronary angiography to examine patients with clinical suspicion of CAD and observed more extensive CAD in diabetic patients as compared to their non diabetic counterparts (16-17). Limited studies have been performed in asymptomatic diabetic patients. MSCT provides accurate non-invasive evaluation of the extent and degree of coronary atherosclerosis and may be used in patients with lower likelihood of CAD. Importantly, excellent sensitivity, specificity and negative predictive values for detection of significant stenosis have also been shown in diabetic patients (7). Thus far, the technique has been used in several studies to explore the presence and extent of CAD in patients with type 2 diabetes. Scholte et al observed a high prevalence of coronary atherosclerosis (80%), which predominantly involved more than 1 coronary artery (74%), in asymptomatic type 2 diabetic patients (12). Obstructive CAD was observed in 26% of patients, similar to observations in the current study. Thus far, no MSCT studies have reported on the presence of CAD in asymptomatic type 1 diabetic patients. However, using magnetic resonance imaging, Kim et al previously evaluated CAD in asymptomatic patients with type 1 diabetes and observed a higher atherosclerotic plaque burden in presence of diabetic nephropathy as compared to normoalbuminuria (6). In that particular study, absence of diabetic nephropathy excluded presence of subclinical obstructive CAD. In our current study, prevalence of nephropathy was low (1%) in type 1 diabetic patients, suggesting that these patients were at relatively low risk. Interestingly however, absence of nephropathy did not exclude subclinical obstructive CAD on MSCT. Nevertheless, when compared to type 2 diabetes, the extent of atherosclerosis was less severe with a lower prevalence of multi-vessel disease and a smaller number of lesions. Moreover, a smaller proportion of patients with type 1 diabetes had obstructive atherosclerosis. Importantly, in this study, presence of type 2 diabetes remained a significant predictor of the severity of atherosclerosis after correction for traditional cardiovascular risk factors including obesity, glycemic control and renal function. Accordingly, the higher atherosclerotic plaque burden in patients with type 2 diabetes may warrant more aggressive anti-atherosclerotic treatment. On

the other hand, MSCT coronary angiography excluded atherosclerosis in approximately 30% of both type 1 and type 2 diabetic patients. This finding raises the question whether anti-atherosclerotic medical therapy should be initiated in all asymptomatic diabetic patients and to which extent. It is possible that MSCT may be used to identify or exclude the presence of atherosclerosis and provide a basis for individually tailored therapy.

Plaque morphology and relation with CAC

In addition to increased severity of atherosclerosis, we also observed differences in relative plaque composition in patients with type 2 diabetes with a higher percentage of non-calcified plaques. This finding is in line with a previous study in which non-calcified plaques comprised 41% of atherosclerotic plaques in asymptomatic patients with type 2 diabetes (12). In retrospective studies, a higher proportion of non-calcified plaques has been associated with unstable CAD. Preliminary prognostic data also suggest that the presence of substantial non-calcified plaque indeed confers worse outcome (18). Accordingly, it has been suggested that these plaques represent more active stages of CAD and may be more prone to rupture (15,19). Notably, the distribution of coronary plaque phenotype has not been previously examined in type 1 diabetes. In contrast with type 2 diabetes, we found a higher proportion of calcified plaques and lower proportion of non-calcified plaques in patients with type 1 diabetes, despite similar CAC scores. As a result, plaque burden was higher for each CAC score category in type 2 diabetes as compared to type 1 diabetes.

Assessment of CAC score has been suggested as a primary step in cardiovascular risk stratification and screening of asymptomatic diabetic patients (20) because the presence of elevated CAC scores has been associated with a higher likelihood of myocardial ischemia (21). However, our current observations suggest that CAC score assessment may be more effective in identifying CAD in patients with type 1 diabetes. Indeed, absence of coronary calcium accurately excluded presence of obstructive coronary atherosclerosis in patients with type 1 diabetes. In contrast, this relation was distorted in patients with type 2 diabetes with a higher prevalence of obstructive CAD in patients without or only minor calcium. As both the prevalence of obstructive CAD and extent of CAD per CAC category were higher, it appears that CAC scores may underestimate CAD in patients with type 2 diabetes, in line with previous comparisons with non-diabetic patients (22). Accordingly, strategies using CAC scores to identify diabetic patients at higher risk should be developed with caution and should potentially be adjusted for type of diabetes.

Study limitations

Several limitations need to be acknowledged. The current analysis was restricted to evaluation of coronary atherosclerosis in type 1 and type 2 diabetes and the pro-atherogenic processes involved in each form of diabetes were not investigated. In addition, the lack of a control group without diabetes should be acknowledged. Because MSCT coronary angiography involves radiation exposure it is not feasible to perform a similar assessment in asymptomatic subjects free of cardiovascular risk. Furthermore, MSCT coronary angiography requires administration of potentially nephrotoxic contrast media, rendering the technique unsuitable for use in asymptomatic diabetic patients with severe renal dysfunction. Finally, no follow-up data were available. Indeed, the prognostic implications of our observations should be evaluated in prospective follow-up studies.

In summary, although CAC scores and prevalence of coronary atherosclerosis were similar between patients with type 1 and type 2 diabetes, CAD was more extensive in the latter. In addition, a relatively higher proportion of non-calcified plaques was observed in type 2 diabetes. These observations may be valuable in the development of targeted management strategies adapted to diabetes type. It is possible that MSCT angiography may be useful to identify or exclude the presence of atherosclerosis and provide a basis for individually tailored therapy.

REFERENCES

1. Morrish NJ, Wang SL, Stevens LK, Fuller JH, Keen H: Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. *Diabetologia* 44 Suppl 2:S14-21, 2001
2. De Backer G, Ambrosioni E, Borch-Johnsen K, Brotons C, Cifkova R, Dallongeville J, Ebrahim S, Faergeman O, Graham I, Mancia G, Manger Cats V, Orth-Gomér K, Perk J, Pyörälä K, Rodicio JL, Sans S, Sansoy V, Sechtem U, Silber S, Thomsen T, Wood D: European guidelines on cardiovascular disease prevention in clinical practice. Third Joint Task Force of European and Other Societies on Cardiovascular Disease Prevention in Clinical Practice. *Eur Heart J* 24:1601-1610, 2003
3. Buse JB, Ginsberg HN, Bakris GL, Clark NG, Costa F, Eckel R, Fonseca V, Gerstein HC, Grundy S, Nesto RW, Pignone MP, Plutzky J, Porte D, Redberg R, Stitzel KF, Stone NJ: American Heart Association; American Diabetes Association: Primary Prevention of Cardiovascular Diseases in People With Diabetes Mellitus. A scientific statement from the American Heart Association and the American Diabetes Association. *Diabetes Care* 30:162-172, 2007
4. Libby P, Nathan DM, Abraham K, Brunzell JD, Fradkin JE, Haffner SM, Hsueh W, Rewers M, Roberts BT, Savage PJ, Skarlatos S, Wassef M, Rabadan-Diehl C: Report of the National Heart, Lung, and Blood Institute-National Institute of Diabetes and Digestive and Kidney Diseases Working Group on Cardiovascular Complications of Type 1 Diabetes Mellitus. *Circulation* 111:3489-3493, 2005
5. Manske CL, Wilson RF, Wang Y, Thomas W: Prevalence of, and risk factors for, angiographically determined coronary artery disease in type I-diabetic patients with nephropathy. *Arch Intern Med* 152:2450-2455, 1992
6. Kim WY, Astrup AS, Stuber M, Tarnow L, Falk E, Botnar RM, Simonsen C, Pietraszek L, Hansen PR, Manning WJ, Andersen NT, Parving HH: Subclinical coronary and aortic atherosclerosis detected by magnetic resonance imaging in type 1 diabetes with and without diabetic nephropathy. *Circulation* 115:228-235, 2007
7. Schuijf JD, Bax JJ, Jukema JW, Lamb HJ, Vliegen HW, Salm LP, de Roos A, van der Wall EE: Noninvasive angiography and assessment of left ventricular function using multislice computed tomography in patients with type 2 diabetes. *Diabetes Care* 27:2905-2910, 2004
8. Achenbach S, Moselewski F, Ropers D, Ferencik M, Hoffmann U, MacNeill B, Pohle K, Baum U, Anders K, Jang IK, Daniel WG, Brady TJ: Detection of calcified and noncalcified coronary atherosclerotic plaque by contrast-enhanced, submillimeter multidetector spiral computed tomography: a segment-based comparison with intravascular ultrasound. *Circulation* 109:14-17, 2003

9. Leber AW, Becker A, Knez A, von Ziegler F, Sirol M, Nikolaou K, Ohnesorge B, Fayad A, Becker CR, Reiser M, Steinbeck G, Boekstegers P: Accuracy of 64-slice computed tomography to classify and quantify plaque volumes in the proximal coronary system: a comparative study using intravascular ultrasound. *J Am Coll Cardiol* 47:672-677, 2006
10. Leber AW, Knez A, Becker A, Becker C, von Ziegler F, Nikolaou K, Rist C, Reiser M, White C, Steinbeck G, Boekstegers P: Accuracy of multidetector spiral computed tomography in identifying and differentiating the composition of coronary atherosclerotic plaques: a comparative study with intracoronary ultrasound. *J Am Coll Cardiol* 43:1241-1247, 2004
11. Rivera JJ, Nasir K, Choi EK, Yoon YE, Chun EJ, Choi SI, Choi DJ, Brancati FL, Blumenthal RS, Chang HJ: Detection of occult coronary artery disease in asymptomatic individuals with diabetes mellitus using non-invasive cardiac angiography. *Atherosclerosis* 203:442-448, 2009
12. Scholte AJ, Schuijf JD, Kharagjitsingh AV, Jukema JW, Pundziute G, van der Wall EE, Bax JJ: Prevalence of coronary artery disease and plaque morphology assessed by multi-slice computed tomography coronary angiography and calcium scoring in asymptomatic patients with type 2 diabetes. *Heart* 94:290-295, 2008
13. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183, 1997
14. Rose G, McCartney P, Reid DD: Self-administration of a questionnaire on chest pain and intermittent claudication. *Br J Prev Soc Med* 31:42-48, 1977
15. Henneman MM, Schuijf JD, Pundziute G, van Werkhoven JM, van der Wall EE, Jukema JW, Bax JJ: Noninvasive evaluation with multislice computed tomography in suspected acute coronary syndrome: plaque morphology on multislice computed tomography versus coronary calcium score. *J Am Coll Cardiol* 52:216-222, 2008
16. Pajunen P, Taskinen MR, Nieminen MS, Syvänne M: Angiographic severity and extent of coronary artery disease in patients with type 1 diabetes mellitus. *Am J Cardiol* 86:1080-1085, 2000
17. Koistinen MJ. Prevalence of asymptomatic myocardial ischaemia in diabetic subjects: *BMJ* 301:92-95, 1990.
18. van Werkhoven JM, Schuijf JD, Gaemperli O, Jukema JW, Boersma E, Wijns W, Stolzmann P, Alkadhi H, Valenta I, Stokkel MPM, Kroft LJ, de Roos A, Pundziute G, Scholte A, van der Wall EE, Kaufmann P, Bax JJ: Prognostic value of multi-slice computed tomography and gated single photon emission computed tomography in patients with suspected coronary artery disease. *J Am Coll Cardiol* 53:623-632, 2009
19. Hoffmann U, Moselewski F, Nieman K, Jang IK, Ferencik M, Rahman AM, Cury RC, Abbara S, Joneidi-Jafari H, Achenbach S, Brady TJ: Noninvasive assessment of plaque morphology and composition in culprit and stable lesions in acute coronary syndrome and stable lesions in stable angina by multidetector computed tomography. *J Am Coll Cardiol* 47:1655-1662, 2006

20. Moser KW, O'Keefe JH, Bateman TM, McGhie IA: Coronary calcium screening in asymptomatic patients as a guide to risk factor modification and stress myocardial perfusion imaging. *J Nucl Cardiol* 10:590–598, 2003
21. Anand DV, Lim E, Hopkins D, Corder R, Shaw LJ, Sharp P, Lipkin D, Lahiri A: Risk stratification in uncomplicated type 2 diabetes: prospective evaluation of the combined use of coronary artery calcium imaging and selective myocardial perfusion scintigraphy. *Eur Heart J* 27:713-721, 2006
22. Raggi P, Shaw LJ, Berman DS, Callister TQ: Prognostic value of coronary artery calcium screening in subjects with and without diabetes. *J Am Coll Cardiol* 43:1663-1669, 2004

CHAPTER 5

Endothelial Dysfunction in Diabetic Patients with Abnormal Myocardial Perfusion in the absence of Epicardial Obstructive Coronary Artery Disease

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ABSTRACT

In patients with diabetes mellitus myocardial perfusion defects are often observed in absence of obstructive epicardial coronary artery disease (CAD), thereby presenting a diagnostic problem. We hypothesized that these perfusion abnormalities may be explained by endothelial dysfunction or occult coronary atherosclerosis.

Methods

Prospectively, 135 asymptomatic patients with diabetes mellitus, underwent cardiovascular screening by coronary artery calcium (CAC) scoring, multislice CT coronary angiography and myocardial perfusion imaging by SPECT. Multislice CT images were evaluated for the presence of obstructive epicardial CAD (≥50% luminal narrowing). To quantify abnormal myocardial perfusion on SPECT images, the summed stress score (SSS) was determined for each patient. The presence of abnormal myocardial perfusion was defined as a SSS of 3 or more. In addition, flow mediated dilatation (FMD) of the brachial artery, a marker of endothelial function, was determined using ultrasonography.

Results

In 35 (27%) patients, obstructive epicardial CAD was observed on multislice CT and these patients were excluded from further analysis. In the remaining 95 patients, abnormal myocardial perfusion was observed in 30 (32%) of patients. FMD was significantly lower in patients with abnormal myocardial perfusion (3.6±2.4%), as compared to those with normal myocardial perfusion (6.4 ± 2.6%) ($P < 0.001$). Importantly, FMD remained a significant predictor of the extent of abnormal myocardial perfusion after correction for cardiovascular risk factors and CAC score ($P < 0.001$). In contrast, no association was observed between non-obstructive plaque burden as reflected by CAC scores and extent of abnormal myocardial perfusion.

Conclusion

In patients with diabetes mellitus, myocardial perfusion abnormalities in absence of obstructive epicardial CAD are associated with endothelial dysfunction.

INTRODUCTION

Coronary artery disease (CAD) constitutes a major cause of morbidity and mortality in patients with diabetes mellitus (DM). In addition, due to diabetic neuropathy myocardial ischemia and infarction may be prevalent in the absence of typical anginal symptoms. Non-invasive assessment of CAD in asymptomatic diabetic patients at higher risk has therefore been previously suggested by the guidelines of the American Diabetes Association and the American Heart Association (1). Those guidelines propose testing for the presence of myocardial ischemia by SPECT as a possible option for early detection and treatment of asymptomatic obstructive CAD. If myocardial perfusion abnormalities are identified, coronary angiography is considered to confirm the presence of clinically relevant obstructive CAD possibly requiring intervention. However, previous studies have shown a discrepancy between the presence of myocardial perfusion defects and obstructive CAD in diabetic patients. Approximately, in 20-40% of diabetic patients myocardial perfusion defects could not be clarified by obstruction of the epicardial coronary arteries (2,3). Also, in the general population, a discrepancy between presence of myocardial perfusion defects and obstructive epicardial CAD has been observed (4-6). Both endothelial dysfunction and occult coronary atherosclerosis were shown to contribute to this condition (4-6). However, the potential mechanisms underlying myocardial perfusion abnormalities not attributable to obstruction of flow in the epicardial coronary arteries have not yet been studied in asymptomatic diabetic patients. Ultrasonographic measurement of the flow mediated dilatation (FMD) of the brachial artery provides non-invasive estimation of systemic endothelial function (7). The observed brachial artery dilatation has been shown to be closely related to coronary endothelial function and vasoreactivity (8,9). The reproducibility of assessment of brachial artery diameter has improved with the development of the wall-track system (WTS) technique, rendering it suitable for cohort studies (10).

The current study was designed to prospectively evaluate the relation between myocardial perfusion by SPECT and endothelial function assessed by FMD in asymptomatic diabetic patients without epicardial obstructive CAD (as evaluated non-invasively by Multi-Slice Computed Tomography (MSCT) coronary angiography). In addition, the association between non-obstructive plaque burden as reflected by coronary artery calcium (CAC) scores and myocardial perfusion was studied.

MATERIALS AND METHODS

Patients and design

Prospectively, 130 asymptomatic diabetic patients were screened for cardiovascular disease. A structured interview, physical examination and laboratory analysis were

acquired. Cardiovascular risk factors were assessed according to the following criteria: Family history of CAD was considered positive if CAD was present in any of the first degree family members. Hypertension was defined as a blood pressure >140/90 mmHg or treatment with antihypertensive medication. Hypercholesterolemia was defined as a total cholesterol level >5.0 mmol/L or use of lipid lowering medication.
Non-invasive MSCT, including CAC scoring and coronary angiography, and myocardial perfusion imaging by SPECT were performed as part of clinical work up in all patients. Concurrent measurement of the brachial FMD was performed in a study setting, approved by the institutional review committee of the Leiden University Medical Center, Leiden.
Primarily, patients not eligible for MSCT coronary angiography due to arrhythmia or contraindications for the use of iodinated contrast media were excluded. Subsequently, those with obstructive epicardial CAD on MSCT angiography were excluded from further analyses. The relation between the presence of myocardial perfusion defects and FMD was thereby assessed in the remainder of patients free of obstructive epicardial CAD.

MSCT data acquisition
Imaging was performed with a 64-slice MSCT scanner (Toshiba Medical Systems, Tokyo, Japan). In patients with a heart rate ≥ 65 beats per minute, oral beta-blocking medication (metoprolol 50 mg or 100 mg) was provided 1 hour preceding the scan, if tolerated. A non-enhanced prospective electrocardiographically gated scan, triggered at 75% of the RR interval with 4 x 3.0 mm collimation was firstly obtained to measure the CAC score and to determine the start and end position of the helical scan.
Subsequently, MSCT angiography was performed using the following parameters: collimation 64 x 0.5 mm, tube rotation time 400, 450 or 500 ms depending on the heart rate, tube current 300 or 350 mA, tube voltage 120 kV. Non-ionic contrast material was administered in an amount of 90–100 ml in the antecubital vein, depending on the total scan time, and with a flow rate of 5 ml/s, followed by 50 ml of saline solution flush. Timing of the scan was determined by automated bolus-tracking in the aortic root. Data were acquired with simultaneous ECG registration during a single breath hold of approximately 10 seconds. Images were reconstructed in the cardiac phase showing least motion artifacts and transferred to a remote workstation (Vitrea 2, Vital Images, Minnetonka, USA) for post-processing.

MSCT data analysis

CAC score

The CAC score was assessed with the application of dedicated software (Vitrea2, Vital Images, USA). CAC was defined as a dense area in the coronary artery exceeding the threshold of 130 Hounsfield units. For each patient the total Agatston score was determined.

Epicardial obstructive CAD

Two experienced observers blinded to patient characteristics interpreted all MSCT coronary angiograms. Discrepancies in interpretation were instantly resolved by consensus. Epicardial coronary arteries were examined by scrolling through axial images, followed by visual assessment of curved multiplanar reconstructions in at least two orthogonal planes. The presence of epicardial obstructive CAD (defined as luminal narrowing ≥ 50% in at least one vessel) was evaluated for each patient.

ECG-gated SPECT data acquisition

During a two-day stress and rest protocol, myocardial perfusion imaging was performed using ECG-gated SPECT with ^{99m}Tc sestamibi (^{99m}TcMIBI). Patients were instructed to abstain from caffeine containing products for 24 hours, preceding the stress test. Stress was induced using intravenous infusion of adenosine at a rate of 140 µg/kg body weight per minute for 6 minutes, accompanied by simultaneous handgrip exercise. After completion of the third minute ^{99m}TcMIBI (500 MBq) was injected intravenously. Blood pressure and a 12-lead ECG were recorded throughout the adenosine infusion. Imaging commenced 120 minutes after radiopharmaceutical injection using a triple-head SPECT gamma camera (GCA 9300/HG; Toshiba Corporation, Tokyo, Japan) equipped with low-energy high-resolution collimators. Images were acquired in accordance with American Society of Nuclear Cardiology (ASNC) guidelines, using a circular 360° orbit, 60 projections, and 40 seconds per projection. Attenuation correction was not applied. Images were processed in the usual manner and short-axis, horizontal long-axis and vertical long-axis views were reconstructed. Patient motion was evaluated by examining the raw cine images.

SPECT data analysis

Short-axis slices were displayed in polar map format, adjusted for peak myocardial activity of 100%. Additional reconstruction yielded standard long- and short-axis projections perpendicular to the heart axis. All views were used for semi-quantitative interpretation. As proposed by the ASNC guidelines, the myocardium was divided

into 17 segments (11). Tracer uptake in each segment was evaluated by two observers in consensus, by use of a 5-point scoring system (0: normal, 1: mild, 2: moderate, 3: severe reduction of tracer uptake and 4: apparent absence of tracer uptake). The total segmental score during stress and rest was used to determine the summed stress score (SSS) and summed rest score (SRS) for each patient. Abnormal myocardial perfusion was defined as SSS ≥3. Patients with a SSS 3-7 were classified as having moderately abnormal myocardial perfusion and those with a SSS ≥8 as having severely abnormal myocardial perfusion. The location of myocardial perfusion abnormalities was classified as apical, anterior, inferior or lateral (11). Finally, regional wall motion on gated SPECT images was analyzed to differentiate between true perfusion abnormalities and attenuation artifacts.

Flow Mediated Dilatation

The brachial artery diameter was evaluated using a Wall Track System (Scanner 200, Pie Medical, Maastricht, the Netherlands), which consisted of an ultrasound imager with a 7.5-MHz linear array transducer connected to a data acquisition system and a personal computer. The principles of this system have been previously described in more detail (12). All measurements were performed by the same experienced sonographer who was unaware of the clinical characteristics of the patients. On the basis of a previous study of 20 healthy middle-aged volunteers, the intra-observer coefficients of variation for FMD assessment by the same sonographer were previously determined to be 0.8% for the baseline diameter of the brachial artery, 2.0% for the maximal diameter, 24.2% for the change in diameter and 24.4% for the calculated FMD. The mean FMD in this group of healthy volunteers was 8.4% ± 2.1%.

In the current study, patients were asked to abstain from medication use, caffeine containing substances and smoking for 24 hours prior to the tests. All measurements were performed on fasting patients, in the morning, in a silent, temperature-controlled clinical research laboratory. Patients had a 15 minute rest in a comfortable supine position and remained in that position throughout the examination. The patients' right arm was placed in extension in the elbow, with the hand in supination, thus eliminating longitudinal movements of the arm. The wrist and elbow were supported by cushions to minimize lateral movements. The heart rate was continuously monitored by a three-lead electrocardiogram. The brachial artery was visualized distal to the elbow. Firstly, three diastolic measurements were averaged to provide a baseline diameter. Thereafter, ischemia was applied to the forearm distal to the location of the transducer by inflation of a blood pressure cuff for 5 minutes at a pressure of 200 mm Hg. After cuff deflation, ultrasonography continued for 5 minutes with measurements at 30-second intervals.

The widest lumen diameter was used as maximal vasodilation. FMD was expressed as percentage change relative to the baseline diameter.

Statistical Analysis

Primarily, patients with obstructive epicardial CAD (≥50% luminal narrowing) on MSCT angiography were identified and excluded from all further analysis.

Continuous variables were expressed as means ± standard deviation. Categorical variables were expressed as number, followed by percentages in parentheses.

To investigate the relationship between FMD and myocardial perfusion, patients were first stratified as having either normal myocardial perfusion (SSS <3) or abnormal myocardial perfusion (SSS ≥3) on SPECT. Average FMD and standard deviation were calculated for both groups, and the independent T-test was applied to evaluate the difference in mean FMD.

To determine the relationship between cardiovascular risk factors and degree of myocardial perfusion abnormalities, a univariate linear regression analysis of baseline characteristics was performed to identify the potential predictors of the number of SSS. Thereafter, multivariate linear regression analysis with backward elimination was performed to identify the independent predictors of the number of SSS.

Finally, the relationship between FMD and prevalence of abnormal myocardial perfusion as well as between the degree non-obstructive atherosclerosis and abnormal myocardial perfusion were evaluated. For this purpose, patients were first categorized according to FMD quartiles and the prevalence of abnormal myocardial perfusion was determined for each FMD quartile. Subsequently, patients were stratified according to CAC category as having a CAC score of 0, 1-10, 11-100 or >100, and the prevalence of abnormal myocardial perfusion was also assessed per CAC category. Influence of decreasing FMD (per quartile) and that of increasing CAC scores (per category) on the prevalence of abnormal myocardial perfusion was investigated.

RESULTS

Patient characteristics

In the total population, MSCT coronary angiography revealed obstructive epicardial CAD (≥50% luminal narrowing) in 35 patients (27%) and these patients were therefore excluded. The patient characteristics of the remaining 95 patients (73%) included in the analysis, are provided in Table 1. Briefly, mean age of this population was 48 ± 12 years and 43 patients (46%) were male.

Table 1. Characteristics of the study population

Age (years)	48 ± 12
Male sex	43 (46%)
Diabetes mellitus duration (years)	15 ± 13
BMI (kg/m2)	28 ± 6
HbA1c (mmol/L)	8.0 ± 1.6
Smoking	23 (25%)
Family history of CAD	42 (45%)
Hypercholesterolemia	62 (66%)
Hypertension	47 (50%)
Type 1- / Type 2 DM	45 /50
Diabetes Treatment	
Diet only	5 (5%)
Oral agent	14 (15%)
Oral agent and insulin	21 (22%)
Insulin	54 (57%)
Cardiovascular medication use	
Statin	41 (43%)
Aspirin	14 (15%)
Ace-inhibitor	15 (16%)
Beta-blocker	9 (9%)
Angiotensin II antagonist	12 (13%)
Calcium channel blocker	7 (7%)
Diureticum	10 (11%)

Data are averages ± standard deviation or numbers (%).
BMI = body mass index, CAD = coronary artery disease.

Baseline imaging findings

Assessment of Myocardial Perfusion by SPECT

The mean SSS was 2.0 ± 3.0 (range 0-13) in patients included in the analysis. In this asymptomatic diabetic population without obstructive epicardial CAD myocardial perfusion abnormalities (SSS ≥3) were observed in 30 patients (32%). Within this group, moderately abnormal myocardial perfusion (SSS 3-7) was revealed in 25 patients (83%); whereas 5 patients (17%) were shown to have severely abnormal myocardial perfusion (SSS ≥8). Furthermore, observed myocardial perfusion abnormalities were reversible in 14 patients (47%), partially reversible in 6 (20%) and fixed in 10 patients (33%). Of note, fixed myocardial perfusion abnormalities mainly comprised of light or moderate decrease in tracer uptake (n = 8). Only 2 patients with a fixed abnormality were shown

to have severe decrease in tracer uptake. In total, 6 patients showed abnormal perfusion apically, 16 patients in the anterior wall, 15 in the inferior wall and 8 in the lateral wall of the myocardium. Overall, 16 patients showed a myocardial perfusion abnormality in a single myocardial wall, 13 patients in two walls and 1 patient in three myocardial walls.

Assessment of Endothelial function by Flow Mediated Dilatation

Mean FMD was 6.0% ± 3.0%, and ranged between 0.0% and 13.3%. The median FMD was calculated to be 5.4% (lower quartile 3.4%, upper quartile 7.3%).

Assessment of CAC score by MSCT

The average CAC score was 60 ± 170. Most patients (n = 53, 55%) had no coronary calcium. The CAC score was found to be in the range 1-10 in 12 patients (13%), 11-100 in 15 patients (16%) and >100 in 15 patients (16%).

Relation between FMD and abnormal myocardial perfusion

In diabetic patients with normal myocardial perfusion the mean FMD was significantly higher (6.4% ± 2.6%) than in patients with abnormal myocardial perfusion (3.6% ± 2.4%) (P <0.001) (Figure 1).

The prevalence of patients with abnormal myocardial perfusion per FMD quartile or CAC score category is provided in Figure 2A. Interestingly, prevalence of myocardial perfusion abnormalities increased gradually from 9% to 61% with decreasing FMD quartiles.

In contrast, no evident trend was observed between the prevalence of abnormal myocardial perfusion and increasing CAC score categories (Figure 2B). While 34% of patients without coronary calcium showed abnormal myocardial perfusion, a similar prevalence (40%) was observed in patients with extensive coronary calcium (CAC>100).

Predictors of the extent of abnormal myocardial perfusion

As illustrated in Table 2, FMD was the only significant predictor of the extent of abnormal myocardial perfusion in a univariate model (P <0.001). Importantly, after correction for other cardiovascular risk factors in a multivariate model with backward elimination, FMD remained a significant predictor of the extent of abnormal myocardial perfusion (P <0.001) (Table 2). HbA1c and hypercholesterolemia were also shown to be independent predictors of the extent of abnormal myocardial perfusion.

DISCUSSION

The current prospective study revealed that 32% of asymptomatic patients with DM had abnormal myocardial perfusion despite the absence of obstructive epicardial CAD.

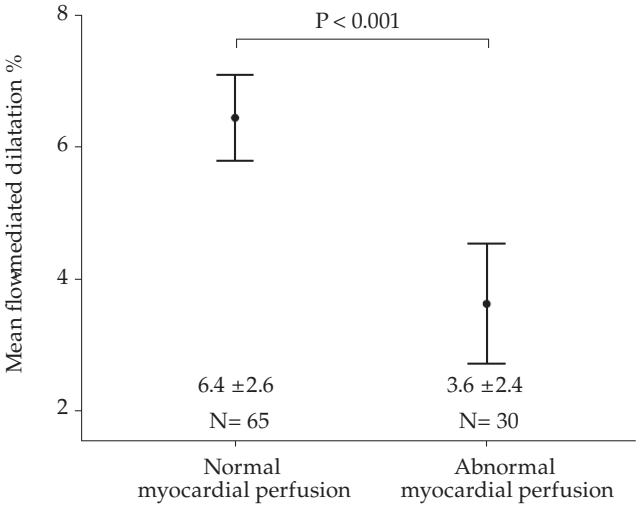

Figure 1. FMD in diabetic patients with normal and abnormal myocardial perfusion. Mean FMD was significantly lower in patients with abnormal myocardial perfusion.

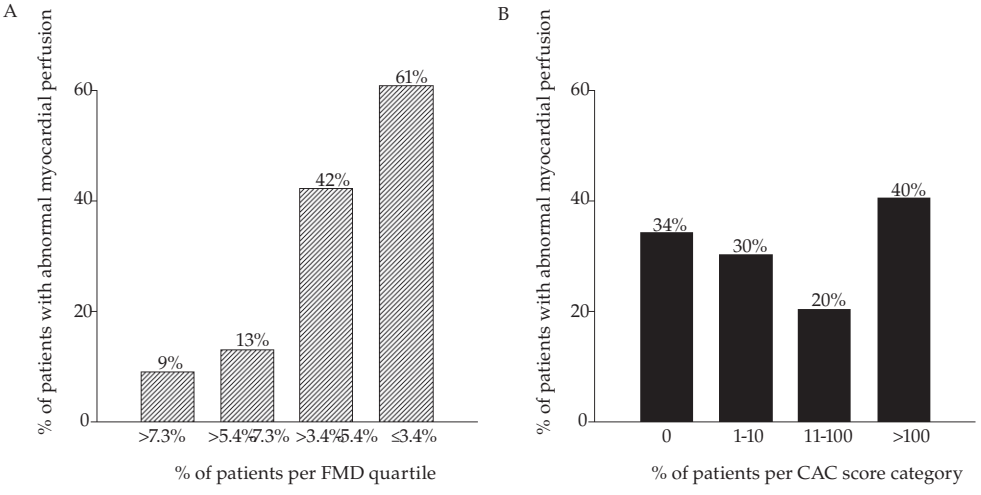

Figure 2. Percentage of patients with abnormal myocardial perfusion per FMD quartile or CAC score category. Prevalence of abnormal myocardial perfusion increased with decreasing FMD (Panel A.), whereas no clear trend was observed among increasing CAC score categories (Panel B.).

Table 2. Predictors of the extent of abnormal myocardial perfusion (SSS) in patients without obstructive epicardial CAD.

	Univariate Analysis		Multivariate Analysis	
	Exp ß (95% CI)	*P Value*	*Exp ß (95% CI)*	*P Value*
FMD (%)	0.61 (0.48-0.78)	<0.001	0.58 (0.43-0.75)	<0.001
CAC score	1.00 (0.99-1.00)	0.22	-	-
Age (years)	1.01 (0.98-1.06)	0.45	-	-
Male sex	1.57 (0.66-3.75)	0.31	-	-
Diabetes Mellitus duration (years)	0.98 (0.95-1.01)	0.33	-	-
BMI (kg/m2)	1.02 (0.95-1.09)	0.59	-	-
HbA1c (mmol/L)	0.76 (0.55-1.04)	0.09	0.50 (0.31-0.82)	0.01
Smoking	0.91 (0.33-2.53)	0.86	-	-
Family history of CAD	0.93 (0.39-2.21)	0.86	0.36 (0.11-1.19)	0.09
Hypercholesterolemia	1.65 (0.64-4.29)	0.30	4.04 (1.07-15.24)	0.04
Hypertension	1.22 (0.51-2.90)	0.66	-	-
Type 1 DM	0.16 (-0.90-1.22)	0.77	-	-
Treatmnet with only diet	1.57 (-0.78-3.91)	0.19	-	-
Treatmnet with oral agents	-0.41 (-1.51-0.68)	0.45	-	-
Treatmnet with insulin	0.15 (-1.18-1.47)	0.83	-	-
Statin use	0.36 (-0.71-1.43)	0.50	-	-
Aspirin use	0.79 (-0.70-2.27)	0.30	-	-
Ace-inhibitor use	-0.14 (-1.59-1.31)	0.85	-	-
Beta-blocker use	0.15 (-1.76-2.05)	0.88	-	-
Angiotensin II antagonist use	-0.99 (-2.57-0.58)	0.21	-	-
Calcium channel blocker use	0.95 (-1.06-2.97)	0.35	-	-
Diureticum use	0.16 (-1.52-1.85)	0.85	-	-

CI = confidence interval, ß = beta coefficient, FMD = flow mediated dilatation, CAC = coronary artery calcium, BMI = body mass index, CAD = coronary artery disease.

Assessment of the brachial FMD showed less vasoreactivity in these patients as compared to those with normal myocardial perfusion. Importantly, after correction for risk factors as well as CAC scores, endothelial function remained a significant predictor of abnormal myocardial perfusion. Further analysis showed the prevalence of myocardial perfusion abnormalities to increase per decreasing FMD quartile, whereas no such trend was observed for occult atherosclerotic plaque burden as determined by CAC scoring.

Myocardial perfusion by SPECT

In the general population of patients with known or suspected CAD, myocardial perfusion imaging (MPI) by SPECT has been shown to successfully identify obstructive CAD and predict future coronary events (13,14). The prognostic value of myocardial

perfusion has also been confirmed in diabetic patients with suspected CAD, as well as in asymptomatic diabetic patients (15-17). Based on these observations in combination with the non-invasive nature of MPI by SPECT, the ADA/AHA has proposed this technique as a potential screening tool for identification of asymptomatic diabetic patients with obstructive CAD (1). However, previous studies in diabetic patients imply that a significant proportion of observed perfusion abnormalities are not attributable to obstruction of blood flow in the epicardial coronary arteries (2,3). In the general population, a similar discrepancy has been observed regarding presentation with anginal symptoms and manifested ischemia in absence of obstructive epicardial CAD (4-6). Mechanisms underlying myocardial perfusion abnormalities in that particular group of patients have been studied extensively and revealed the presence of diffuse coronary atherosclerosis and endothelial dysfunction as potential causal factors (4-6). However, little is known about the contribution of these parameters to the occurrence of myocardial perfusion defects in asymptomatic patients with DM. Therefore, in the current study we examined the influence of endothelial dysfunction and non-obstructive atherosclerotic plaque burden on myocardial perfusion in asymptomatic diabetic patients without epicardial obstructive CAD.

Assessment of endothelial function by FMD

Assessment of systemic endothelial function by FMD has been shown to be closely related to coronary vasoreactivity (8,9). To date, measurement of brachial FMD is accepted as the most validated and reproducible non-invasive technique for assessment of endothelial function (7). Inter-observer reproducibility of the baseline and maximum post-ischemia brachial artery are satisfactory with diameter variations of approximately 4%, and have improved with the introduction of semi-automated wall track systems (10,18). In contrast, the intra-observer reproducibility is limited by within subject variability and surrounding factors, as vasoreactivity fluctuates through the day and is further influenced by stress, temperature, diet and glucose levels (10). Therefore, FMD appears to be less applicable for individual serial testing. However, a panel of experts has recognized the test as appropriate for cohort studies, providing that patient and surrounding factors are standardized (19). FMD ranges from approximately 20% in young adults to 0% in patients with established CAD (20). In diabetic patients, mean FMD values tend to be lower as well (range 0-12%) (20). In DM, endothelial dysfunction is suspected to be induced by hyperglycemia (sorbitol-, hexosamin-, protein kinase C-, and advanced glycation end product-pathways) and insulin resistance, which result in mitochondrial superoxide overproduction. As a consequence, nitric oxide availability is negatively affected, leading to endothelial dysfunction (21,22).

Relationship with myocardial perfusion

In the current study, FMD of the brachial artery was further decreased in diabetic patients with abnormal myocardial perfusion. Several studies have described an association between endothelial dysfunction and the occurrence of myocardial ischemia due to impaired endothelium-dependent coronary vasodilation during stress (23). It is therefore presumable that in the current study insufficient vasomotor response in the coronary microvasculature due to endothelial dysfunction may indeed have resulted in relative hypoperfusion during stress (reflected by reversible perfusion abnormalities on SPECT) in the absence of obstructive epicardial CAD (23). However, endothelial dysfunction has also been shown to affect resting myocardial perfusion (24). Accordingly, hypoperfusion of the microvasculature during rest may occur. Interestingly, in our study the majority of fixed perfusion abnormalities comprised of a mild decrease in tracer uptake, which has been linked to hypoperfused but viable myocardial tissue rather than scar (25). Nevertheless, impaired coronary endothelial function has also been suggested as a cause of myocardial infarction in patients with minimally obstructive disease, possibly through prolonged vasoconstriction (26). Therefore, it is possible that the fixed abnormalities comprising of severely decreased tracer uptake, which we observed in 2 patients, may indeed reflect loss of viable myocardium. Finally, the possibility remains that some of the fixed perfusion abnormalities were due to attenuation. However, in the current study, regional wall motion on gated SPECT images was analyzed for optimal differentiation between true perfusion abnormalities and attenuation artifacts.

Importantly, FMD was shown to be an independent predictor of the extent of abnormal perfusion, even after correction for occult coronary atherosclerosis by means of CAC scores. Accordingly, it appears that in the absence of epicardial obstructive CAD, endothelial dysfunction may have a greater impact on myocardial perfusion than the extent of diffuse atherosclerosis. Further investigations are needed to confirm these observations.

Potential clinical implications

The direct cardiovascular prognostic consequence of endothelial dysfunction in diabetic patients remains to be determined. In previous studies of non-diabetic patients, impaired endothelial function has been shown to predict cardiovascular events (27). In addition, Bugiardini and colleagues have shown reduced coronary vasoreactivity in angiographically normal coronaries to be a predecessor of overt atherosclerosis on angiography after a long term follow-up of 10 years (28). Therefore, dysfunction of the endothelium seems to occur in early stages of vascular disease, and may be reversible. Accordingly, cholesterol lowering therapy has shown to improve endothelium dependent vasomotion in patients with hypercholesterolemia, as well as myocardial

perfusion in a non-diabetic cohort of patients with previous evidence of myocardial perfusion defects on SPECT (29,30). In line with these observations, inducible ischemia was shown to resolve in 79% of asymptomatic diabetic patients who had abnormal myocardial perfusion at baseline in the DIAD study (31). This recovery was associated with intensification of treatment with statins, aspirin and ACE inhibitors.

Thus, in the current study abnormal myocardial perfusion not attributable to obstructive CAD was related with impaired endothelial function. As this condition is most likely to be an early stage of vascular disease amendable by treatment, intensification of anti-atherogenic therapy seems to be indicated in asymptomatic diabetic patients with perfusion abnormalities even in absence of obstructive epicardial CAD.

Furthermore, considering that in DM abnormal myocardial perfusion is often observed in the absence of epicardial obstructive CAD, the question emerges whether this test should not be accompanied by a non-invasive anatomical test such as MSCT coronary angiography. Indeed, referral of all asymptomatic diabetic patients with abnormal myocardial perfusion for invasive coronary angiography should be avoided considering that many patients will not have obstructive epicardial stenosis amendable for revascularization. Accordingly, combined non-invasive imaging of myocardial perfusion imaging by SPECT and coronary anatomy with MSCT may allow a more comprehensive and accurate assessment of CAD and facilitate further management. However, routine combination of these two non-invasive imaging modalities carries the disadvantage of increased costs and radiation exposure. Possibly, MSCT coronary angiography could be of most incremental value in asymptomatic diabetic patients with abnormal myocardial perfusion, to differentiate between obstructive epicardial CAD and endothelial dysfunction as a causal factor.

Study Limitations

Firstly, the size of the study population was limited. In addition, it must be acknowledged that brachial FMD is a measure of systemic endothelial function, and not a direct measure of coronary endothelial function. However, previous studies have shown a good correlation between brachial FMD and direct invasive measures of coronary endothelial function (13,14). Furthermore, the radiation burden associated with combined MSCT (64-slice MSCT approximately 9-15 mSv) and SPECT myocardial perfusion imaging (^{99m}TcMIBI approximately 7 mSv per scan) is a limitation (32,33). However, the radiation dose of MSCT has been decreased significantly with the use of dedicated dose reduction MSCT acquisition techniques that have recently become available (34,35). Finally, no follow-up data were available and whether our observations have prognostic implications should be evaluated in prospective follow-up studies.

CONCLUSION

In asymptomatic diabetic patients with no obstructive epicardial CAD, abnormal myocardial perfusion is common and is strongly associated with impaired endothelial function. In view of the likely reversible nature of endothelial dysfunction and the cardiovascular prognostic value of abnormal myocardial perfusion in asymptomatic diabetic patients, it is presumable that asymptomatic diabetic patients with abnormal myocardial perfusion will benefit from intensified pharmacological treatment even in the absence of obstructive epicardial CAD.

REFERENCES

1. Consensus development conference on the diagnosis of coronary heart disease in people with diabetes: 10-11 February 1998, Miami, Florida. American Diabetes Association. *Diabetes Care*. 1998;21:1551-1559.
2. Scholte AJ, Schuijf JD, Kharagjitsingh AV, et al. Different manifestations of coronary artery disease by stress SPECT myocardial perfusion imaging, coronary calcium scoring, and multislice CT coronary angiography in asymptomatic patients with type 2 diabetes mellitus. *J Nucl Cardiol*. 2008;15:503-509.
3. Kang X, Berman DS, Lewin H, et al. Comparative ability of myocardial perfusion single-photon emission computed tomography to detect coronary artery disease in patients with and without diabetes mellitus. *Am Heart J*. 1999;137:949-957.
4. Bellamy MF, Goodfellow J, Tweddel AC, Dunstan FD, Lewis MJ, Henderson AH. Syndrome X and endothelial dysfunction. *Cardiovasc Res*. 1998;40:410-417.
5. Masci PG, Laclaustra M, Lara JG, Kaski JC. Brachial artery flow-mediated dilation and myocardial perfusion in patients with cardiac syndrome X. *Am J Cardiol*. 2005;95:1478-1480.
6. Rodés-Cabau J, Candell-Riera J, Angel J, et al. Relation of myocardial perfusion defects and nonsignificant coronary lesions by angiography with insights from intravascular ultrasound and coronary pressure measurements. *Am J Cardiol*. 2005;96:1621-1626.
7. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation*. 2007;115:1285-1295.
8. Anderson TJ, Uehata A, Gerhard MD, et al. Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol*. 1995;26:1235-1241.
9. Pellegrino T, Storto G, Filardi PP, et al. Relationship between brachial artery flow-mediated dilation and coronary flow reserve in patients with peripheral artery disease. *J Nucl Med*. 2005;46:1997-2002.
10. Hijmering ML, Stroes ES, Pasterkamp G, Sierevogel M, Banga JD, Rabelink TJ. Variability of flow mediated dilation: consequences for clinical application. *Atherosclerosis*. 2001;157:369-373.
11. Hansen CL, Goldstein RA, Akinboboye OO, et al. American Society of Nuclear Cardiology. Myocardial perfusion and function: single photon emission computed tomography. *J Nucl Cardiol*. 2007;14:e39-60.
12. Hoeks AP, Willekes C, Boutouyrie P, Brands PJ, Willigers JM, Reneman RS. Automated detection of local artery wall thickness based on M-line signal processing. *Ultrasound Med Biol*. 1997;23:1017-1023.
13. Iskandrian AS, Verani MS. Exercise perfusion imaging in coronary artery disease: Physiology and diagnosis. In: Davis FA, *Nuclear Cardiac Imaging: Principles and Applications*. Philadelphia, 1996:73-143.

14. Thomas GS, Miyamoto MI, Morello AP 3rd, et al. Technetium 99m sestamibi myocardial perfusion imaging predicts clinical outcome in the community outpatient setting. The Nuclear Utility in the Community (NUC) Study. *J Am Coll Cardiol*. 2004;43:213-223.
15. Zellweger MJ, Hachamovitch R, Kang X, et al. Prognostic relevance of symptoms versus objective evidence of coronary artery disease in diabetic patients. *Eur Heart J*. 2004;25:543-550.
16. Valensi P, Pariès J, Brulport-Cerisier V, et al. Predictive value of silent myocardial ischemia for cardiac events in diabetic patients: influence of age in a French multicenter study. *Diabetes Care*. 2005;28:2722-2727.
17. Elhendy A, Huurman A, Schinkel AF, et al. Association of ischemia on stress (99m)Tc-tetrofosmin myocardial perfusion imaging with all-cause mortality in patients with diabetes mellitus. *J Nucl Med*. 2005;46:1589-1595.
18. De Roos NM, Bots ML, Schouten EG, Katan MB. Within-subject variability of flow-mediated vasodilation of the brachial artery in healthy men and women: implications for experimental studies. *Ultrasound Med Biol*. 2003;29:401-406.
19. Corretti MC, Anderson TJ, Benjamin EJ, et al. International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*. 2002;39:257-265.
20. Bots ML, Westerink J, Rabelink TJ, de Koning EJ. Assessment of flow-mediated vasodilatation (FMD) of the brachial artery: effects of technical aspects of the FMD measurement on the FMD response. *Eur Heart J*. 2005;26:363-368.
21. Creager MA, Lüscher TF, Cosentino F, Beckman JA. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part I. *Circulation*. 2003;108:1527-1532.
22. Tan KC, Chow WS, Ai VH, Metz C, Bucala R, Lam KS. Advanced glycation end products and endothelial dysfunction in type 2 diabetes. *Diabetes Care*. 2002;25:1055-1059.
23. Hasdai D, Gibbons RJ, Holmes DR Jr, Higano ST, Lerman A. Coronary endothelial dysfunction in humans is associated with myocardial perfusion defects. *Circulation*. 1997;96:3390-3395.
24. Johnson NP, Gould KL. Clinical evaluation of a new concept: resting myocardial perfusion heterogeneity quantified by markovian analysis of PET identifies coronary microvascular dysfunction and early atherosclerosis in 1,034 subjects. *J Nucl Med*. 2005;46:1427-1437.
25. Dakik HA, Howell JF, Lawrie GM et al. Assessment of myocardial viability with 99mTc-sestamibi tomography before coronary bypass graft surgery: correlation with histopathology and postoperative improvement in cardiac function. *Circulation*. 1997;96:2892-2898.
26. Lerman A, Zeiher AM. Endothelial function: cardiac events. *Circulation*. 2005;111:363-368.

27. Gokce N, Keaney JF Jr, Hunter LM, et al. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J Am Coll Cardiol*. 2003;41:1769-1775.
28. Bugiardini R, Manfrini O, Pizzi C, Fontana F, Morgagni G. Endothelial function predicts future development of coronary artery disease: a study of women with chest pain and normal coronary angiograms. *Circulation*. 2004;109:2518-2523.
29. Egashira K, Hirooka Y, Kai H, et al. Reduction in serum cholesterol with pravastatin improves endothelium-dependent coronary vasomotion in patients with hypercholesterolemia. *Circulation*. 1994;89:2519-2524.
30. Mostaza JM, Gomez MV, Gallardo F, et al. Cholesterol reduction improves myocardial perfusion abnormalities in patients with coronary artery disease and average cholesterol levels. *J Am Coll Cardiol*. 2000;35:76-82.
31. Wackers FJ, Chyun DA, Young LH, et al. Detection of Ischemia in Asymptomatic Diabetics (DIAD) Investigators. Resolution of asymptomatic myocardial ischemia in patients with type 2 diabetes in the Detection of Ischemia in Asymptomatic Diabetics (DIAD) study. *Diabetes Care*. 2007;30:2892-2898.
32. Einstein AJ, Moser KW, Thompson RC, Cerqueira MD, Henzlova MJ. Radiation dose to patients from cardiac diagnostic imaging. *Circulation*. 2007;116:1290-1305.
33. Einstein AJ. *Heart*. 2008;94:1519-1521.
34. Husmann L, Valenta I, Gaemperli O, et al. Feasibility of low-dose coronary CT angiography: first experience with prospective ECG-gating. *Eur Heart J*. 2008;29:191-197.
35. Rybicki FJ, Otero HJ, Steigner ML, et al. Initial evaluation of coronary images from 320-detector row computed tomography. *Int J Cardiovasc Imaging*. 2008;24:535-546.

PART II

Cardiovascular risk assessment in diabetes

CHAPTER 6

Non-invasive Cardiac Imaging Techniques and Vascular Tools for the Assessment of Cardiovascular Disease in Type 2 Diabetes Mellitus

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ABSTRACT

Cardiovascular disease is the major cause of mortality in type 2 diabetes mellitus. The criteria for the selection of those asymptomatic patients with type 2 diabetes who should undergo cardiac screening and the therapeutic consequences of screening remain controversial.

Non-invasive techniques as markers of atherosclerosis and myocardial ischaemia may aid risk stratification and the implementation of tailored therapy for the patient with type 2 diabetes. In the present article we review the literature on the implementation of non-invasive vascular tools and cardiac imaging techniques in this patient group. The value of these techniques as endpoints in clinical trials and as risk estimators in asymptomatic diabetic patients is discussed.

Carotid intima–media thickness, arterial stiffness and flow-mediated dilation are abnormal long before the onset of type 2 diabetes. These vascular tools are therefore most likely to be useful for the identification of 'at risk' patients during the early stages of atherosclerotic disease. The additional value of these tools in risk stratification and tailored therapy in type 2 diabetes remains to be proven.

Cardiac imaging techniques are more justified in individuals with a strong clinical suspicion of advanced coronary heart disease (CHD). Asymptomatic myocardial ischaemia can be detected by stress echocardiography and myocardial perfusion imaging. The more recently developed non-invasive multi-slice computed tomography angiography is recommended for exclusion of CHD, and can therefore be used to screen asymptomatic patients with type 2 diabetes, but has the associated disadvantages of high radiation exposure and costs. Therefore, we propose an algorithm for the screening of asymptomatic diabetic patients, the first step of which consists of coronary artery calcium score assessment and exercise ECG.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality in type 2 diabetes mellitus [1]. Current guidelines on the treatment of dyslipidaemia and hypertension in diabetes recommend rigorous primary prevention, with target lipid and blood pressure levels similar to those used for secondary prevention in non-diabetic patients [2]. To date, there is much debate as to whether all diabetic patients will benefit from this strategy and whether risk stratification should be attempted.

Non-invasive imaging techniques as markers of atherosclerosis and myocardial ischaemia may help risk stratification and the implementation of tailored therapy for the individual patient. However, many of these tools have not been validated in diabetic individuals. In this article we will review the reproducibility and predictive value of the following surrogate markers of atherosclerosis: intima–media thickness (IMT), arterial stiffness and flow-mediated dilation (FMD). We will discuss the diagnostic accuracy and predictive value of imaging techniques used for direct anatomic assessment of coronary atherosclerosis (coronary artery calcium [CAC] scores and multi-slice computed tomography [MSCT] angiography) and functional tests that detect myocardial ischaemia (ambulatory ECG, exercise ECG, stress echocardiography (SE) and nuclear myocardial perfusion imaging (MPI) by single photon emission computed tomography (SPECT). Finally, the value of these non-invasive techniques as endpoints in clinical trials and as risk estimators in diabetic patients will be discussed. We will concentrate on methods of risk stratification and the implementation of non invasive techniques in patients with type 2 diabetes, as the value of these techniques has scarcely been studied in type 1 diabetes.

SURROGATE MARKERS OF ATHEROSCLEROSIS

Carotid intima media thickness

Since its introduction in the early 1990s, IMT, especially carotid IMT (CIMT), has increasingly been used as a surrogate marker of atherosclerotic disease. IMT can be assessed non-invasively using B-mode ultrasound. Two approaches are used: (1) multiple measurements of CIMT in the near and far walls of the three main segments of the carotid arteries (common carotid, bifurcation and internal carotid); and (2) automated computerised measurement of CIMT, restricted to the far wall of the distal common carotid artery. Computerised measurement of CIMT is superior in terms of precision and reproducibility, with an approximately 3% difference between two successive measurements [3]. As a result, common CIMT has become a valid tool for large-scale multicentre studies. However, the common carotid artery is less likely to have intrusive plaque than the bifurcation and internal segments of the carotid arteries.

CIMT correlates with prevalent CVD and with risk factors for CVD [4]. In prospective studies, CIMT has proven to be a consistent and independent predictor for coronary events and stroke in the general population [5-6].

CIMT in type 2 diabetes Mean common CIMT in middle-aged individuals is reported to range from 0.71–0.98 mm in diabetic patients vs 0.66–0.85 mm in controls [7-9]. In diabetic individuals without a history of myocardial infarction CIMT is similar to that in non-diabetic individuals with a history of myocardial infarction [9]. Progression of maximal CIMT in the Insulin Resistance Atherosclerosis Study was twice as high in persons with diabetes vs controls [10], but other studies report lower rates [11]. In type 2 diabetes, prevalent CVD is associated with higher CIMT [9]. In two prospective studies, baseline CIMT was shown to be an independent predictor of cardiovascular events [12-13]. However, when Folsom and colleagues analysed CIMT in a large cohort that included 1500 diabetic participants, they found that CIMT has predictive value for future coronary events only in combination with several other novel risk factors [14].

CIMT measurements show good reproducibility. CIMT is increased in type 2 diabetic patients with CVD and is an independent predictor of coronary events. However, the magnitude of its predictive value when added to other risk factors is questionable.

Arterial stiffness

Whereas IMT is a marker of structural vessel wall properties, arterial stiffness reflects functional wall properties. Stiffness can be measured in many ways, including distensibility, pulse wave velocity (PWV) and augmentation index (AIx). Distensibility, defined as the change in arterial lumen diameter during the cardiac cycle, can be evaluated by ultrasound imaging using wall-tracking systems based on Doppler shift or using B- or M-mode. The change in arterial diameter during the cardiac cycle varies by about 5-6% in middle-aged individuals [15]. PWV is the speed with which the arterial pressure wave progresses through the arterial tree, and this increases with increasing vascular stiffness. The PWV can be determined either by placing a probe on two sites and recording the waveform simultaneously, or by recording the waveforms independently and comparing the time delay at both sites with a simultaneously measured QRS complex. PWV gradually increases with age, from about 4 m/s in the third decade to 10 m/s in the ninth decade. The AIx, which is the augmentation of aortal pressure as a percentage of pulse pressure, has also emerged as a parameter for arterial stiffness (Fig. 1) [16-17]. Studies report excellent reproducibility of PWV, with a coefficient of

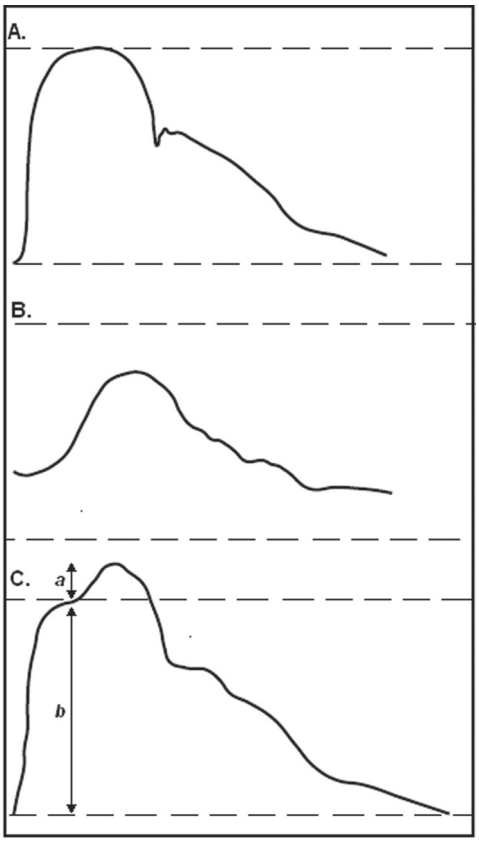

Figure 1. The pulse pressure wave form. (*a)* The incident wave generated by the left ventricle (in the ascending aorta). (*b*) Waves reflected back from the peripheral vascular bed (ascending aorta). (*c*) The resultant wave in the ascending aorta, which is a combination of (*a)* and (*b*). AIx is the measure of additional pressure to which the left ventricle is subjected as a result of wave reflection and is calculated as: AIx = (a/[b+a]) × 100

variation (CV) of approximately 3.2%, which is lower than that for distensibility indices (CV 5.3%) or AIx (CV 10.1%) [17-19].

In cross-sectional studies, arterial stiffness is strongly associated with age and classical risk factors for CVD [15, 20-21], and it has been reported to be related to angiographic coronary atherosclerosis [17]. In a cohort of men aged >70 years, baseline arterial distensibility predicted cardiovascular mortality during a 2 year follow-up, but added little to clinical risk estimation [22]. However, in a Danish population study, aortic PWV predicted a composite of cardiovascular events outcome above and beyond traditional risk factors [23].

Arterial stiffness in type 2 diabetes Diabetic patients have increased arterial stiffness [17, 24]. Compromised carotid distensibility and PWV have been demonstrated even before the onset of diabetes, in patients with impaired glucose tolerance. Healthy offspring of type 2 diabetic patients have a higher PWV than matched controls [17, 25]. Arterial stiffness in diabetes is related to prevalent CVD [16] and has shown to be an independent predictor of CHD [26].
Baseline distensibility did not predict mortality in 140 individuals with impaired glucose tolerance during a follow-up period of 6.6 years [18]. Conversely, PWV does seem to have a reasonable predictive value for mortality in patients with impaired glucose tolerance and type 2 diabetes [24].

The reproducibility of PWV is superior to that of AIx and distensibility. Therefore, PWV is the most accepted method for estimating vascular stiffness. Vascular stiffness is increased in type 2 diabetic patients with CVD and has been shown to predict cardiovascular mortality.

Flow mediated dilation
FMD of the brachial artery is a non-invasive technique for measuring endothelial function. FMD is measured with B-mode ultrasound or a wall-track system. The brachial artery is visualised in the elbow, and by inflating a cuff (mostly distal to the elbow) for 4 min, hypoxia is created. After deflation, reactive hyperaemia induces shear stress, thereby stimulating NO synthesis, resulting in NO-dependent dilation [27]. FMD is thus defined as the percentage change in the diameter of the brachial artery after hypoxia, estimated to be 5–10% in healthy individuals. The observed brachial artery dilatation has shown to be closely related to coronary vasoreactivity [28].
FMD fluctuates during the day and is influenced by the temperature, stress, diet, glucose levels and the menstrual cycle [29]. Within-subject variability of FMD is therefore often poor, with coefficients of variation ranging from 14-50% [29-30]. In spite of the biological variation, there is good intra- and interobserver reproducibility for measurements of baseline and maximum post-ischaemia diameter in the brachial artery (diameter variations of approximately 4%) [30].
FMD ranges from about 10% in young adults to 0% in patients with established coronary heart disease (CHD), and it has proven to be predictive for the presence of CHD [31] and for future coronary events in high-risk populations [32]. High sensitivity and high negative predictive values were calculated using cut-off points of 8.1-10% [32]. FMD has not been independently associated with coronary events in patients at lower risk [33].

FMD in type 2 diabetes Type 2 diabetes is associated with endothelial dysfunction. The underlying mechanisms are suspected to be related to hyperglycaemia (sorbitol, hexosamine, protein kinase C, and AGE pathways) and insulin resistance, which results in mitochondrial superoxide overproduction, and thus decreased NO availability [34-35]. Clustering of risk factors such as dyslipidaemia, hypertension and obesity in the metabolic syndrome play an additional role. Insulin-mediated vasodilatation is at least in part NO-dependent, thus explaining how insulin resistance may cause endothelial dysfunction.

The predictive value of endothelial dysfunction in epicardial coronary arteries of diabetic patients has been established for long-term coronary events [36]. However, to our knowledge, no studies to date have evaluated the relationship between FMD and prediction of coronary events in diabetes.

FMD is a marker of endothelial function. It should only be assessed under strictly constant external and physical circumstances, so that reproducibility is optimised. The potential of FMD for the identification of type 2 diabetic patients at risk for CVD is as yet unknown.

DIRECT ANATOMIC ASSESSMENT OF CORONARY ATHEROSCLEROSIS

Coronary artery calcium scores

Anatomical and intravascular studies have illustrated that the presence of coronary calcium is indicative of coronary atherosclerosis [37]. Coronary calcification can be detected non-invasively by electron beam CT (EBCT), and more recently by MSCT. Agatston et al. developed a coronary calcium scoring algorithm, based on calcification volume and density, that is now widely used in clinical practice [38]. The extent of coronary calcium increases with age, and is, on average, higher in men than in women [39-40].

CAC scores in type 2 diabetes Diabetic patients without manifest CVD have a higher CAC score than non-diabetic individuals, independent of classical risk factors [41-43]. In addition, CAC scores show significantly more progression over time in patients with diabetes than in non-diabetic patients [44].

In a study by Raggi et al. [45], 10,377 patients (903 with diabetes) were followed for a period of 5.0 ± 3.5 years after CAC imaging. Mortality increased with increasing baseline CAC levels for both diabetic and non-diabetic individuals. However, despite similar CAC scores, there was a greater increase in mortality in diabetic than non-diabetic patients

for every increase in CAC score [45]. The predictive value of CAC scores in diabetes has been questioned by Qu et al. [46], who found no significant relationship between coronary events and CAC scores during a 6 year follow-up of 269 diabetic patients [46].

CAC score is associated with prevalent CVD in diabetes. However, CAC scores may underestimate the risk for CVD in type 2 diabetic patients.

Multislice CT coronary angiography

Application of MSCT scanners for non-invasive coronary angiography has developed rapidly during the recent years. Employment of 16 and 64 slice systems have demonstrated a sensitivity ranging from 83–99% and a specificity of between 93% and 98% [47-51]. Several studies have demonstrated that CT angiography has a high negative predictive value of 99% on average [47-51]. Therefore, the technique is currently most suited to exclude CHD.

Besides visualisation of the coronary artery lumen (Fig. 2), CT angiography allows the identification of non-stenotic atherosclerosis and the various types of plaques. In addition, chronic myocardial infarction and left ventricular ejection fraction can be assessed. Non-stenotic atherosclerosis may prove to be a predictor of coronary events; however, this remains to be determined in prospective long-term clinical studies. Plaques can be classified as non-calcified, mixed or calcified. Initial comparisons have shown that calcification may represent the duration of atherosclerosis, whereas non-calcified and mixed lesions are more frequently observed in patients with an acute coronary syndrome [52].

MSCT is subject to a number of limitations, including exposure to a relatively high dose of radiation, currently in the range of 9-12 mSv [47,51], lower accuracy in the presence of severe calcification and movement artefacts, and limited application possibilities in case of irregular heart rate [49-51]. Taking the radiation exposure and the high negative predictive value of MSCT angiography into consideration, this technique is recommended for excluding CHD in patients of intermediate risk.

MSCT coronary angiography in type 2 diabetes MSCT angiography has demonstrated a higher percentage of non-calcified and calcified plaques and a relatively lower percentage of mixed plaques in diabetes [53], which can be explained by the rapid progression of atherosclerosis. Schuijf et al., have reported a sensitivity and specificity of 95% for detection of stenosis. Inclusion of uninterpretable segments reduced sensitivity and specificity to 81% and 82%, respectively [54]. In an evaluation of the diagnostic accuracy of 16 slice MSCT angiography, there were no statistically significant differences between

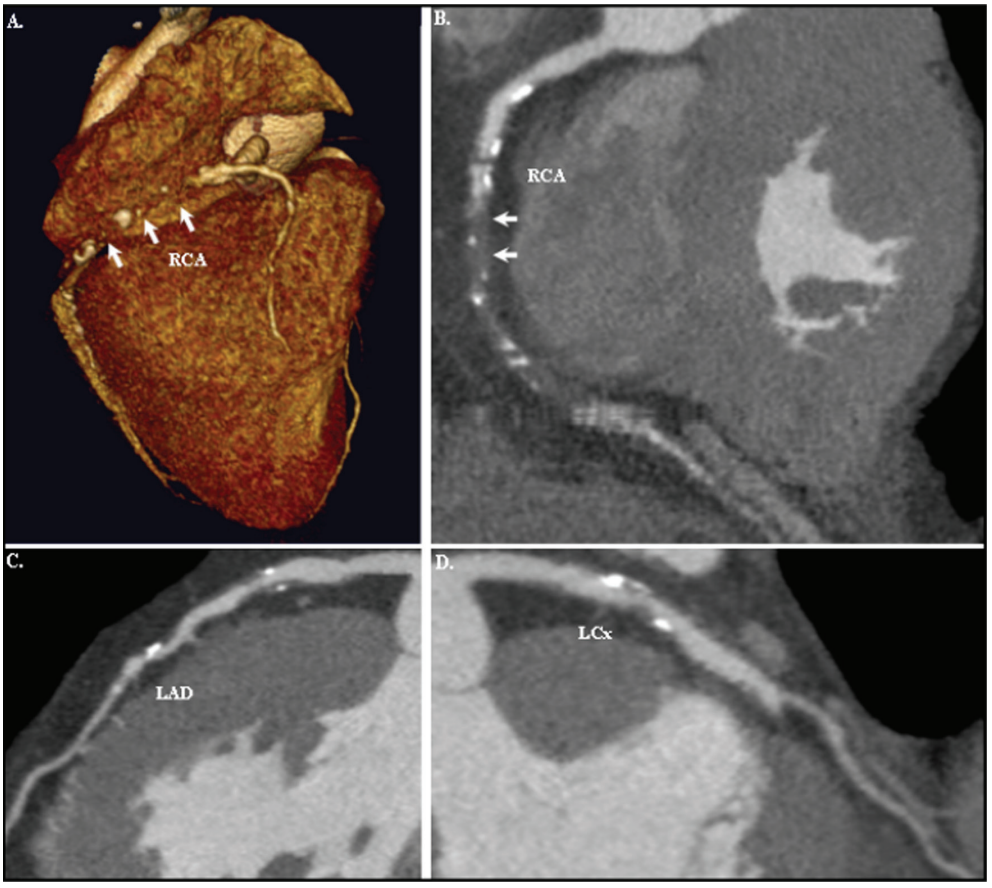

Figure 2. An asymptomatic patient with type 2 diabetes was screened for CAD using MSCT angiography. (*a)* The occluded right coronary artery (RCA) is easily visible using the three-dimensional volume rendering technique, which provides an overview of coronary anatomy. Arrows indicate occlusion. (*b*) Multiplanar reconstruction of the RCA gives a more precise overview of abnormalities. (*c*), (*d)* Multiplanar reconstruction of the left anterior descending (LAD) and left circumflex (LCx) coronary arteries

the diabetic and non-diabetic individuals in the study population [55]. Importantly, negative predictive value of MSCT angiography in diabetes was found to be 98% and 100% on segmental and patient basis, respectively [55].

The prevalence of CHD has been assessed by MSCT angiography in 70 asymptomatic patients with type 2 diabetes. The majority of the patients (80%) had atherosclerosis (obstructive CHD [luminal narrowing ≥50%] in 26%, non-obstructive CHD in 54% of

patients) [56]. Thus, results on the use of non-invasive MSCT angiography for CHD screening and as a prognostic indicator in the diabetic population appear promising, but further studies in larger population groups are needed.

MSCT angiography has good sensitivity, specificity and negative predictive value for identification of CHD in diabetic patients. However, assessment of CHD by MSCT in asymptomatic type 2 diabetic patients should be limited to patients at high risk, because of exposure to high radiation and contrast as well as cost factors.

FUNCTIONAL TESTS IN ASSESSMENT OF CORONARY ARTERY DISEASE

Functional tests detect myocardial ischemia which is the physiologic consequence of coronary obstruction. These include: ambulatory ECG, exercise ECG, stress echocardiography and nuclear myocardial perfusion imaging.

Ambulatory ECG

It has been postulated that periods of silent myocardial ischaemia (SMI), which can be detected with ambulatory ECG, precede a first coronary event. Ambulatory ECG monitoring can be performed with a three-channel recording system for a continuous period of 48 h. Transient myocardial ischaemia is defined as the presence of episodes showing >0.1 mV horizontal or downsloping ST-segment depression. The sensitivity of ambulatory ECG for detecting CHD is poor, ranging from 19–62% [57-59]. Compared with coronary angiography, the specificity of ambulatory ECG ranged between 54% and 92% [57-60]. Frequent episodes of transient ischaemia detected by ambulatory ECG have shown to be a marker for an increased coronary event rate in asymptomatic middle-aged men and in patients with known CHD [61].

Ambulatory ECG in type 2 diabetes The prevalence of SMI in diabetes as assessed by ambulatory ECG varies between 35% and 58% [62-64]. Although the prevalence of SMI determined by this method is expected to be higher in diabetic than non-diabetic individuals, findings have been inconsistent. Comparison of diabetic and non-diabetic patients in the Asymptomatic Cardiac Ischemia Pilot (ACIP) study, illustrated lower rates of asymptomatic ischaemia in diabetes, despite more extensive and diffuse coronary disease in the non-diabetic group [65]. A study comparing exercise ECG with ambulatory ECG for detection of SMI in diabetes reported that ambulatory ECG identified ischaemia only in diabetic patients with three-vessel disease, whereas exercise ECG also revealed ischemia in one- and two-vessel disease [66]. In one study, patients with previously

detected silent ischaemia had a higher incidence of new coronary events (87%) than those with no silent ischaemia (51%) during a 40 month follow-up period [63]. Further studies are needed to validate the prognostic value of SMI detected by ambulatory ECG.

The diagnostic value of ambulatory ECG for CHD is poor. The predictive value of ischaemia detected by ambulatory ECG in type 2 diabetic patients has not been extensively studied.

Exercise ECG

The exercise ECG is considered positive for myocardial ischaemia if horizontal downsloping or upsloping ST-segment depression of ≥0.1 mV occurs at least 0.08 s after the J point. In a pooled meta-analysis of 24,074 patients who had undergone both an exercise ECG and conventional coronary angiography, mean sensitivity and specificity were calculated to be 68% and 77%, respectively, for the diagnosis of CHD by exercise ECG [67]. Sensitivity was higher in three-vessel disease [67]. In addition to myocardial ischaemia, the exercise ECG provides information on exercise capacity and haemodynamic response, which both have prognostic value [68].

The prognostic significance of exercise-induced myocardial ischaemia has been evaluated in prospective studies [69-70]. In a population-based study, an average follow-up period of 10 years was completed in 1,769 asymptomatic men who had undergone an exercise ECG [69]. The risks of acute coronary events and cardiac death were increased 1.7-fold and 3.5-fold, respectively, in men with SMI compared with men without SMI, after adjusting for conventional factors.

Exercise ECG in type 2 diabetes mellitus The use of an exercise ECG for diagnosing myocardial ischaemia specifically in the setting of diabetes has not been assessed in large studies. In an evaluation of the correlation between the ECG exercise test and coronary angiography for the identification of significant coronary artery stenosis in 59 diabetic patients, the sensitivity and specificity were 75% and 77%, respectively [71]. The mean positive predictive value of the exercise ECG for predicting angiographic coronary disease varies between 70% and 90% [72-73]. However, the test is often inconclusive or unfeasible in diabetic patients (approximately 32%) because exercise capability may be impaired by peripheral vascular or neuropathic disease [72]. Furthermore, the specificity of this method is lower for detecting significant coronary artery disease in diabetes because of the presence of microvascular disease.

Abnormal ECG stress tests have shown to be independent predictors of coronary events [74-75]. A 38 month follow-up of 262 asymptomatic diabetic patients who had undergone a maximal ECG stress test showed a good negative predictive value (97%) for major cardiac endpoints [74]. Gerson et al. [75] showed that the exercise ECG successfully identified all diabetic patients who developed clinical CHD within 50 months, but provided little prognostic information after the first 50 months, suggesting the need for serial testing.

Exercise ECG has moderate sensitivity and specificity for detection of CHD. During intermediate follow-up exercise ECG has shown to have a good predictive value of CE. Application of exercise ECG as a screening tool in type 2 diabetes is limited as the test is often inconclusive.

Stress echocardiography

SE is a well-established functional technique for assessing CHD that can be used to demonstrate inducible wall motion abnormalities in the general population. Exercise or a pharmacological form of stress can be used. In the case for the former, echocardiography is performed shortly after exercise. This method provides additional information on exercise capacity, symptoms and haemodynamic response, which are beneficial prognostic factors. A potential hindrance may be the rapid resolution of ischaemia after exercise, and therefore normalisation of any wall motion abnormality prior to echocardiography. Pharmacologically induced stress echocardiography is preferred in those with a limited exercise capacity. An additional advantage is that images are obtained during stress. In a meta-analysis of 10,817 patients in which dobutamine was compared with stress testing with adenosine or dipyridamole, dobutamine echocardiography had the highest combination of sensitivity (80%) and specificity (84%) for the diagnosis of coronary disease [76]. The accuracy of the method is dependent on the degree of stenosis, the amount of myocardium at risk and the degree of induced wall motion abnormality [77]. False-negative results are more likely with submaximal exercise (in the case of exercise-induced stress), single-vessel disease and moderate stenosis (50–70%) [78].

The presence of ischaemia on SE and the number of ischaemic segments predict the likelihood of coronary events during long-term follow-up among members of the general population with known or suspected CHD [79-80]. However, in a 10 year follow-up of 1,832 asymptomatic patients who underwent SE, exercise testing and a resting echocardiogram, SE did not offer additional prognostic information in terms of identifying patients at a higher risk of coronary events [81].

SE in type 2 diabetes The diagnostic accuracy of SE for significant CHD in diabetes has been verified in two studies. In one study in which 55 diabetic patients underwent dobutamine SE and invasive angiography, the sensitivity and specificity of SE were 81% and 85%, respectively [82]. Another study that compared SE with coronary angiography in diabetic patients (n=52) reported a similar sensitivity (82%) but a much lower specificity (54%) [83].

In a prospective study, SE plus an exercise ECG were used to screen 71 diabetic patients with unknown asymptomatic cardiac disease and two or more cardiovascular risk factors [84]. Those who obtained an abnormal result in one test underwent coronary angiography, and if necessary, revascularisation. Compared with patients randomised to the control arm (n=70), coronary events were significantly reduced in the screening arm during follow-up [84]. The preclinical diagnosis of CHD by SE may therefore be effective. However, more studies are needed to determine the prognostic role of SE in screening for cardiac disease in asymptomatic diabetic patients.

The sensitivity and specificity of SE for diagnosing extensive CHD are satisfactory. However, the predictive value of a positive test in type 2 diabetes needs to be further analysed.

SPECT myocardial perfusion imaging

The majority of studies on ischaemia have used SPECT MPI. This imaging modality reveals the presence and extent of perfusion defects. Images are taken following exposure to stress (exercise of pharmacological) and at rest, allowing the identification of fixed and reversible defects (Fig. 3). The dimensions of the left ventricle and ejection fraction can also be determined. An analysis of the diagnostic accuracy of pharmacologically induced stress MPI in a pooled meta-analysis of 10,817 patients with angiographic data reported a mean sensitivity and specificity of 88% and 77%, respectively [85].

Perfusion defects are significant predictors of coronary events in patients with known or suspected CHD [86]. However, over a follow-up period of 4.6 years the presence of perfusion defects did not independently predict coronary events in a purely asymptomatic group of volunteers [87]. Normal MPI results have shown a low coronary event rate (1%) over a 5 year follow-up period [88]. Significant predictors of future coronary events after pharmacologically induced stress MPI include large defects, defects in multiple coronary artery territory suggestive of multi-vessel disease, major irreversible defects, left ventricular dilatation and decreased resting left ventricular ejection fraction [86].

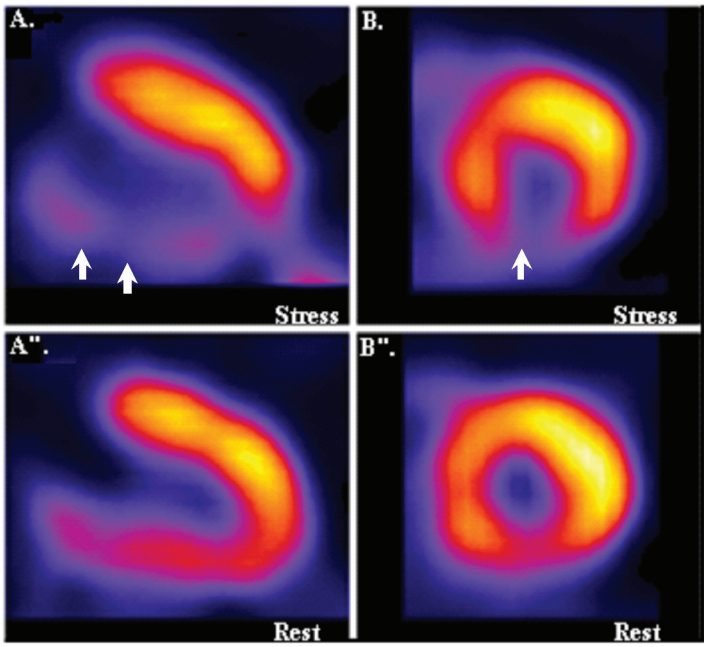

Figure 3. Myocardial perfusion imaging was carried out in the patient described in Fig. 2, in whom coronary abnormalities had been observed on MSCT angiography. (*a)* A perfusion defect was observed in the posterolateral segment (indicated by the arrows) during stress, which did not exist during rest (*b*), indicating ischaemia. (*c*) Partial ischaemia was observed during stress, shown by an increase in the size of the defect in the inferior segment (indicated by the arrow) compared with the rest scan (*d)*

Nuclear SPECT MPI in type 2 diabetes To our knowledge, the diagnostic accuracy of MPI in diabetes has only been studied by Kang et al. [89], who performed MPI and conventional coronary angiography n 138 diabetic patients. Mean sensitivity and specificity were 86% and 56%, respectively, for ≥50% coronary stenosis, and 90% and 50% for ≥70% coronary stenosis [89].

In asymptomatic diabetic patients, the rate of SMI diagnosed by stress MPI ranges from 17–59% (Table 1) [90-95]. In general, a higher percentage of perfusion defects has been detected in retrospective studies [90-91]. In the Detection of Ischemia in Asymptomatic Diabetics (DIAD) study, which included 1,123 participants, the occurrence of perfusion defects was not significantly associated with the traditional risk factors for CVD [92].

During an intermediate follow-up period, persistent and reversible perfusion defects have been shown to be predictors of coronary events in asymptomatic diabetic patients [93-95]. Rajagopalan et al. [90], categorised diabetic patients, according to SPECT imaging

Table 1. Comparison of studies which have used single-photon emission-computed tomography myocardial perfusion imaging to detect *silent* ischemia in diabetic patients

Study group	No. of patients	Patient characteristics	Study nature	Abnormal results (%)	Other details
Rajagopalan et al. [90]	1,427	No known cardiac history Patients with abnormal resting ECG included	Retrospective	58% abnormal scans 18% high-risk scans (high risk: SSS ≤47)	High-risk scans were associated with ECG Q waves, PAD, HbA_{1c}, male sex, age, LDL-cholesterol
Miller et al. [91]	1,738	No known cardiac history Patients with abnormal resting ECG included	Retrospective	59% abnormal scans	High-risk scans in 19.7%
Wackers et al. [92] (DIAD study)	522	No known cardiac history Patients with abnormal resting ECG excluded	Prospective	22% abnormal results (out of which, 73% abnormal scans and 37% other abnormalities)	Abnormal test result was not associated with traditional cardiac risk factors; 50% of patients were incapable of exercise
Sultan et al. [93]	419	No known cardiac history Besides DM, ³1 traditional cardiac risk factor Patients with abnormal resting ECG included	Prospective	17% abnormal scans (abnormal: defect in ≥3/20 segments)	Male sex, triacylglycerol, low creatinine clearance, HbA_{1c}>8% were independent predictors of abnormal scans
Zellweger et al. [94]	826	No known cardiac history	Prospective	39% abnormal scans (abnormal: SSS <4 or SDS ≥2)	
Valensi et al. [95]	370	No known cardiac history Besides DM, ³2 traditional cardiac risk factors Patients with abnormal resting ECG excluded	Prospective	26% abnormal scans	Silent ischaemia was associated with higher age and triacylglycerol and lower HDL levels

DM, diabetes; PAD, peripheral arterial disease; SDS = summed difference score; SSS, summed stress score

scans, as being at high, intermediate or low risk. The annual mortality rate was 5.9%, 5.0% and 3.6%, respectively, with a significant difference in mortality ($p<0.001$) between the three groups [90]. The long-term prognostic value of MPI in asymptomatic diabetic patients needs to be further analysed. It is speculated that concurrent abnormalities of perfusion imaging scans in diabetic patients with normal coronary angiograms may be caused by microangiopathy or endothelial dysfunction, and therefore represent an increased likelihood of future coronary events [96].

MPI shows good sensitivity but poor specificity (possibly because of microvascular disease) for diagnosing CHD in diabetes. Intermediate follow-up has shown a good predictive value of MPI for coronary events in type 2 diabetes.

CONCLUSION

CIMT, arterial stiffness and perhaps FMD are abnormal long before the onset of diabetes. Therefore these measurements are the most likely to be useful for the identification of at risk patients during the early stages of atherosclerotic disease, when functional wall properties are still reversible. However, further studies are necessary to evaluate whether these tools provide any additional prognostic value when used in combination with clinical risk scores (Table 2) before they can be implemented on large scale in clinical practice.

In individuals with a strong clinical suspicion of advanced CHD, cardiac imaging techniques are more warranted. When functional techniques are compared, ambulatory ECG and exercise ECG are less sensitive and specific than functional cardiac imaging tests for the detection of ischaemia in type 2 diabetes. Head-to-head comparison has revealed that SPECT MPI has a higher sensitivity than SE for the detection of multi-vessel and single-vessel CHD [97]. Furthermore, the predictive value of SPECT MPI in the diabetic population has been studied more extensively than that of SE (Table 2). CAC scoring and the more recently developed MSCT non-invasive coronary angiography allow quantification of atherosclerotic burden. CAC scores have been shown to predict coronary events [56]. MSCT coronary angiography has good sensitivity for the identification of prevalent CHD and can therefore enable more widespread screening in combination with CAC scores in diabetes, but its use is limited by radiation exposure and costs.

We propose an algorithm for the screening of asymptomatic diabetic patients (Fig. 4). A selection strategy using a CAC score >100 AU has been shown to be an effective way of identifying patients with moderate to large perfusion defects [98]. Nevertheless, recent observations have shown that low CAC scores do not exclude CHD in diabetes

Table 2. Comparison of various non-invasive vascular tools and cardiac imaging techniques *(for references see text)*

Tool/technique	Reproducibility	Detection of prevalent CAD		Prediction of CAD events		Details
		Non-DM2	*DM2*	*Non-DM2*	*DM2*	
1. Vascular tools						
IMT	Good: variability <5%	++ [4]	+ [9]	++ [5, 6]	+ [12-14]	
Vascular stiffness	Mediocre: variability 11–15%	++ [17]	+ [16, 26]	+ [22, 23]	+ [18, 24]	
FMD	Poor: variability up to 50%	+ [31]	Unknown	± [32, 33]	Unknown	High intersession variability
2. Anatomical tests						
CAC scores	Good	++ [37]	++ [56]	++ [100]	± [45, 46] Limited studies	
MSCT angiography	Good	++ [47-51]	++ [54-56]	Unknown	Unknown	High radiation doses
3. Functional tests						
Ambulatory ECG	Unknown	± [57-60] Reasonable sensitivity Low specificity	± [65, 66] Low sensitivity	+ [61]	± [63] Limited studies	
Exercise ECG	Unknown	+ [67] Reasonable sensitivity Reasonable specificity	+ [71-73] Reasonable sensitivity Low specificity	+ [69, 70]	+ [74, 75]	Not feasible in 32% of patients with DM2
Nuclear MPI	Good	+ [85] Good sensitivity Reasonable specificity	+ [89] Good sensitivity Low specificity	++ [86-88]	++ [90, 93-95] Based on intermediate follow-up	More long-term follow-up studies in DM2 are needed
SE	Good	+ [76-78] Good sensitivity Good specificity	+ [82, 83] Limited studies Good sensitivity Good specificity	± [79-81]	± [84] Limited studies	Relatively high false-negative rate in single-vessel disease and moderate stenosis

++, strong and consistent association in several studies in multivariate analysis; +, association in most studies in multivariate analysis; ±, association in some studies or association only in univariate analysis DM2, type 2 diabetes

[56]. Based on this, the initial step of our algorithm involves the combined use of CAC assessment and exercise ECG to maximise sensitivity for the detection of CHD. MPI or MSCT coronary angiography seem to be justified for individuals with a CAC score >100 or a positive exercise ECG. Conventional coronary angiography can then be considered in the presence of ischaemia according to stress MPI or obstructive atherosclerosis illustrated by MSCT angiography. Prospective studies may be conducted to evaluate the effectiveness of such a screening approach.

The criteria for the selection of those asymptomatic patients with type 2 diabetes who should undergo non-invasive cardiac screening for risk stratification remain controversial. The 'two or more risk factors' criterion for screening, as suggested by the 1998 American Diabetes Association guidelines, failed to accurately identify a large number of patients with ischaemia in the DIAD study [92]. Future studies may prove non-invasive vascular tools such as measurement of CIMT, PWV and FMD to be more effective for the identification of patients at risk who should be screened for CHD (Fig. 4).

THE FUTURE

In type 2 diabetic patients, plaque development is not only accelerated but also distinct, exhibiting more lipid-rich atheroma, macrophage infiltration and a higher thrombogenic potential compared with development in non-diabetic individuals [99]. This implies that screening tools such as magnetic resonance angiography, which enable assessment of plaque composition and may reflect the real culprit, i.e. plaque vulnerability, could emerge as more potent risk predictors in diabetes. However, the application of magnetic resonance angiography as a screening tool is not feasible in the near future because of the high costs and complex methodology involved.

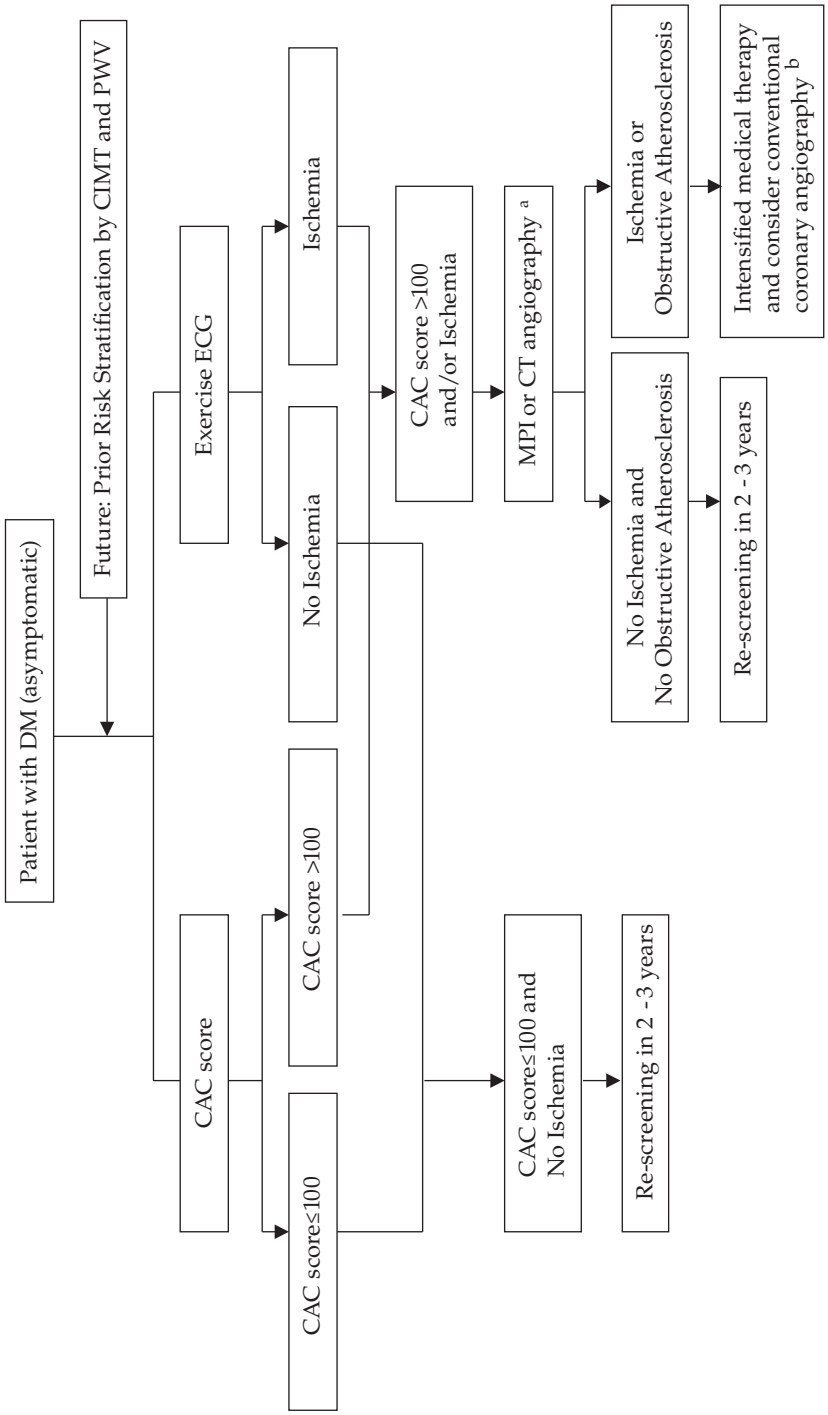

Figure 4. Proposed algorithm for the screening of asymptomatic diabetic patients. [a]Choice of test according to availability and patient characteristics (in patients with severely impaired kidney function or atrial fibrillation, CT angiography should be avoided). [b]Conventional coronary angiography can be considered in the presence of obstructive atherosclerosis in a proximal segment of a coronary artery or extensive ischaemia

REFERENCES

1. Morrish NJ, Wang SL, Stevens LK, Fuller JH, Keen H (2001) Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. Diabetologia 44(Suppl 2): S14-21
2. Buse JB, Ginsberg HN, Bakris GL et al. (2007) Primary prevention of cardiovascular diseases in people with diabetes mellitus. A scientific statement from the American Heart Association and the American Diabetes Association. Diabetes Care 30:162-172
3. Graf S, Gariepy J, Massonneau M et al. (1999) Experimental and clinical validation of arterial diameter waveform and intimal media thickness obtained from B-mode ultrasound image processing. Ultrasound Med Biol 25: 1353-1363
4. Burke GL, Evans GW, Riley WA et al. (1995) Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study. Stroke 26: 386-391
5. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr (1999) Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 340: 14-22
6. Chambless LE, Heiss G, Folsom AR et al. (1997) Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987–1993. Am J Epidemiol 146: 483-494
7. Niskanen L, Rauramaa R, Miettinen H, Haffner SM, Mercuri M, Uusitupa M (1996) Carotid artery intima-media thickness in elderly patients with NIDDM and in nondiabetic subjects. Stroke 27: 1986-1992
8. Bonora E, Kiechl S, Oberhollenzer F et al. (2000) Impaired glucose tolerance, type II diabetes mellitus and carotid atherosclerosis: prospective results from the Bruneck Study. Diabetologia 43: 156-164
9. Lee CD, Folsom AR, Pankow JS, Brancati FL; Atherosclerosis Risk in Communities (ARIC) Study Investigators (2004) Cardiovascular events in diabetic and nondiabetic adults with or without history of myocardial infarction. Circulation 109: 855-860
10. Wagenknecht LE, Zaccaro D, Espeland MA, Karter AJ, O'Leary DH, Haffner SM (2003) Diabetes and progression of carotid atherosclerosis: the Insulin Resistance Atherosclerosis Study. Arterioscler Thromb Vasc Biol 23: 1035-1041
11. van der Meer IM, Iglesias del Sol A, Hak AE, Bots ML, Hofman A, Witteman JC (2003) Risk factors for progression of atherosclerosis measured at multiple sites in the arterial tree: the Rotterdam Study. Stroke 34: 2374-2379
12. Bernard S, Sérusclat A, Targe F et al. (2005) Incremental predictive value of carotid ultrasonography in the assessment of coronary risk in a cohort of asymptomatic type 2 diabetic subjects. Diabetes Care 28: 1158-1162

13. Yamasaki Y, Kodama M, Nishizawa H et al. (2000) Carotid intima-media thickness in Japanese type 2 diabetic subjects: predictors of progression and relationship with incident coronary heart disease. Diabetes Care 23: 1310-1315
14. Folsom AR, Chambless LE, Duncan BB, Gilbert AC, Pankow JS; Atherosclerosis Risk in Communities Study Investigators (2003) Prediction of coronary heart disease in middle-aged adults with diabetes. Diabetes Care 26: 2777-2784
15. Benetos A, Laurent S, Hoeks AP, Boutouyrie PH, Safar ME (1993) Arterial alterations with aging and high blood pressure. A noninvasive study of carotid and femoral arteries. Arterioscler Thromb 13: 90-97
16. Fukui M, Kitagawa Y, Nakamura N et al. (2003) Augmentation of central arterial pressure as a marker of atherosclerosis in patients with type 2 diabetes. Diabetes Res Clin Pract 59: 153-161
17. Weber T, Auer J, O'Rourke MF et al. (2004) Arterial stiffness, wave reflections, and the risk of coronary artery disease. Circulation 109: 184-189
18. Henry RM, Kostense PJ, Spijkerman AM et al. (2003) Arterial stiffness increases with deteriorating glucose tolerance status: the Hoorn Study. Circulation 107: 2089-2095
19. Wilkinson IB, Fuchs SA, Jansen IM et al. (1998) Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. J Hypertens 16: 2079-84
20. Lakatta EG, Levy D (2003) Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a 'set up' for vascular disease. Circulation 107: 139-146
21. Simons PC, Algra A, Bots ML, Grobbee DE, van der Graaf Y (1999) Common carotid intima-media thickness and arterial stiffness: indicators of cardiovascular risk in high-risk patients. The SMART Study (Second Manifestations of ARTerial disease). Circulation 100: 951-957
22. Störk S, van den Beld AW, von Schacky C et al. (2004) Carotid artery plaque burden, stiffness, and mortality risk in elderly men: a prospective, population-based cohort study. Circulation 110: 344-348
23. Willum-Hansen T, Staessen JA, Torp-Pedersen C et al. (2006) Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation 113: 664-670
24. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG (2002) Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation 106: 2085-2090
25. Schram MT, Henry RM, van Dijk RA et al. (2004) Increased central artery stiffness in impaired glucose metabolism and type 2 diabetes: the Hoorn Study. Hypertension 43: 176-181

26. Hatsuda S, Shoji T, Shinohara K et al. (2006) Regional arterial stiffness associated with ischemic heart disease in type 2 diabetes mellitus. J Atheroscler Thromb 13: 114-121
27. Joannides R, Haefeli WE, Linder L et al. (1995) Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. Circulation 91: 1314-1319
28. Anderson TJ, Uehata A, Gerhard MD et al. (1995) Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 26: 1235-1241
29. Hijmering ML, Stroes ES, Pasterkamp G, Sierevogel M, Banga JD, Rabelink TJ (2001) Variability of flow mediated dilation: consequences for clinical application. Atherosclerosis 157: 369-373
30. De Roos NM, Bots ML, Schouten EG, Katan MB (2003) Within-subject variability of flow-mediated vasodilation of the brachial artery in healthy men and women: implications for experimental studies. Ultrasound Med Biol 29: 401-406
31. Schroeder S, Enderle MD, Ossen R et al. (1999) Noninvasive determination of endothelium-mediated vasodilation as a screening test for coronary artery disease: pilot study to assess the predictive value in comparison with angina pectoris, exercise electrocardiography, and myocardial perfusion imaging. Am Heart J 138: 731-739
32. Gokce N, Keaney JF Jr, Hunter LM et al. (2003) Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. J Am Coll Cardiol 41: 1769-1775
33. Fathi R, Haluska B, Isbel N, Short L, Marwick TH (2004) The relative importance of vascular structure and function in predicting cardiovascular events. J Am Coll Cardiol 43: 616-623
34. Creager MA, Lüscher TF, Cosentino F, Beckman JA (2003) Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part I. Circulation 108: 1527-1532
35. Tan KC, Chow WS, Ai VH, Metz C, Bucala R, Lam KS (2002) Advanced glycation end products and endothelial dysfunction in type 2 diabetes. Diabetes Care 25: 1055-1059
36. Nitenberg A, Pham I, Antony I, Valensi P, Attali JR, Chemla D (2005) Cardiovascular outcome of patients with abnormal coronary vasomotion and normal coronary arteriography is worse in type 2 diabetes mellitus than in arterial hypertension: a 10 year follow-up study. Atherosclerosis 183: 113-120
37. Schmermund A, Baumgart D, Görge G et al. (1998) Measuring the effect of risk factors on coronary atherosclerosis: coronary calcium score versus angiographic disease severity. J Am Coll Cardiol 31: 1267-1273
38. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R (1990) Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 15: 827-832

39. Allison MA, Wright CM (2005) Age and gender are the strongest clinical correlates of prevalent coronary calcification (R1). Int J Cardiol 98: 325-330
40. Elkeles RS, Feher MD, Flather MD et al. (2004) The association of coronary calcium score and conventional cardiovascular risk factors in type 2 diabetic subjects asymptomatic for coronary heart disease (The PREDICT Study). Diabet Med 21: 1129-1134
41. Hoff JA, Quinn L, Sevrukov A et al. (2003) The prevalence of coronary artery calcium among diabetic individuals without known coronary artery disease. J Am Coll Cardiol 41: 1008-1012
42. Schurgin S, Rich S, Mazzone T (2001) Increased prevalence of significant coronary artery calcification in patients with diabetes. Diabetes Care 24: 335-338
43. Reaven PD, Sacks J; Investigators for the VADT (2005) Coronary artery and abdominal aortic calcification are associated with cardiovascular disease in type 2 diabetes. Diabetologia 48: 379-385
44. Raggi P, Cooil B, Ratti C, Callister TQ, Budoff M (2005) Progression of coronary artery calcium and occurrence of myocardial infarction in patients with and without diabetes mellitus. Hypertension 46: 238-243
45. Raggi P, Shaw LJ, Berman DS, Callister TQ (2004) Prognostic value of coronary artery calcium screening in subjects with and without diabetes. J Am Coll Cardiol 43: 1663-1669
46. Qu W, Le TT, Azen SP et al. (2003) Value of coronary artery calcium scanning by computed tomography for predicting coronary heart disease in diabetic subjects. Diabetes Care 26: 905-910
47. Mollet NR, Cademartiri F, Krestin GP et al. (2005) Improved diagnostic accuracy with 16-row multi-slice computed tomography coronary angiography. J Am Coll Cardiol 45: 128-132
48. Hoffmann MH, Shi H, Schmitz BL et al. (2005) Noninvasive coronary angiography with multislice computed tomography. JAMA 293: 2471-2478
49. Raff GL, Gallagher MJ, O'Neill WW, Goldstein JA (2005) Diagnostic accuracy of noninvasive coronary angiography using 64-slice spiral computed tomography. J Am Coll Cardiol 46: 552-557
50. Mollet NR, Cademartiri F, van Mieghem CA et al. (2005) High-resolution spiral computed tomography coronary angiography in patients referred for diagnostic conventional coronary angiography. Circulation 112: 2318-2323
51. Ropers D, Rixe J, Anders K et al. (2006) Usefulness of multidetector row spiral computed tomography with 64- × 0.6-mm collimation and 330-ms rotation for the noninvasive detection of significant coronary artery stenoses. Am J Cardiol 97: 343-348
52. Hoffmann U, Moselewski F, Nieman K et al. (2006) Noninvasive assessment of plaque morphology and composition in culprit and stable lesions in acute coronary syndrome and

stable lesions in stable angina by multidetector computed tomography. J Am Coll Cardiol 47: 1655-1662

53. Pundziute G, Schuijf JD, Jukema JW et al. (2007) Noninvasive assessment of plaque characteristics with multislice computed tomography coronary angiography in symptomatic diabetic patients. Diabetes Care 30: 1113-1119
54. Schuijf JD, Bax JJ, Jukema JW et al. (2004) Noninvasive angiography and assessment of left ventricular function using multislice computed tomography in patients with type 2 diabetes. Diabetes Care 27: 2905-2910
55. Schuijf JD, Mollet NR, Cademartiri F et al. (2006) Do risk factors influence the diagnostic accuracy of noninvasive coronary angiography with multislice computed tomography? J Nucl Cardiol 13: 635-641
56. Scholte AJ, Schuijf JD, Kharagjitsingh AV et al. (2008) Prevalence of coronary artery disease and plaque morphology assessed by multi-slice computed tomography coronary angiography and calcium scoring in asymptomatic patients with type 2 diabetes. Heart 94:290-295
57. Crawford MH, Mendoza CA, O'Rourke RA, White DH, Boucher CA, Gorwit J (1978) Limitations of continuous ambulatory electrocardiogram monitoring for detecting coronary artery disease. Ann Intern Med 89: 1-5
58. Ochotny R, Luczak D, Górski L, Błaszczyk K, Jasek S, Koźbiał H (1992) 24-Hour ECG monitoring by the Holter system in early diagnosis of coronary disease Pol Arch Med Wewn 87: 265-270 (article in Polish)
59. Nair CK, Khan IA, Esterbrooks DJ, Ryschon KL, Hilleman DE (2001) Diagnostic and prognostic value of Holter-detected ST-segment deviation in unselected patients with chest pain referred for coronary angiography: a long-term follow-up analysis. Chest 120: 834-839
60. Quyyumi A, Crake T, Wright C, Mockus L, Fox K (1987) The role of ambulatory ST-segment monitoring in the diagnosis of coronary artery disease: comparison with exercise testing and thallium scintigraphy. Eur Heart J 8: 124-129
61. Sajadieh A, Nielsen OW, Rasmussen V, Hein HO, Hansen JF (2005) Prevalence and prognostic significance of daily-life silent myocardial ischaemia in middle-aged and elderly subjects with no apparent heart disease. Eur Heart J 26: 1402-1409
62. Chiariello M, Indolfi C, Cotecchia MR, Sifola C, Romano M, Condorelli M (1985) Asymptomatic transient ST changes during ambulatory ECG monitoring in diabetic patients. Am Heart J 110: 529-534
63. Aronow WS, Mercando AD, Epstein S (1992) Prevalence of silent myocardial ischemia detected by 24-hour ambulatory electrocardiography, and its association with new coronary events at 40-month follow-up in elderly diabetic and nondiabetic patients with coronary artery disease. Am J Cardiol 69: 555-556

64. Marín Huerta E, Rayo I, Lara JI et al. (1989) Silent myocardial ischemia during Holter monitoring in patients with diabetes mellitus. Rev Esp Cardiol 42: 519-529 (article in Spanish)
65. Caracciolo EA, Chaitman BR, Forman SA et al. (1996) Diabetics with coronary disease have a prevalence of asymptomatic ischemia during exercise treadmill testing and ambulatory ischemia monitoring similar to that of nondiabetic patients. An ACIP database study. ACIP Investigators. Asymptomatic Cardiac Ischemia Pilot Investigators. Circulation 93: 2097-2105
66. Ahluwalia G, Jain P, Chugh SK, Wasir HS, Kaul U (1995) Silent myocardial ischemia in diabetics with normal autonomic function. Int J Cardiol 48: 147-153
67. Gianrossi R, Detrano R, Mulvihill D et al. (1989) Exercise-induced ST depression in the diagnosis of coronary artery disease. A meta-analysis. Circulation 80: 87-98
68. Roger VL, Jacobsen SJ, Pellikka PA, Miller TD, Bailey KR, Gersh BJ (1998) Prognostic value of treadmill exercise testing: a population-based study in Olmsted County, Minnesota. Circulation 98: 2836-2841
69. Laukkanen JA, Kurl S, Lakka TA et al. (2001) Exercise-induced silent myocardial ischemia and coronary morbidity and mortality in middle-aged men. J Am Coll Cardiol 38: 72-79
70. Giagnoni E, Secchi MB, Wu SC et al. (1983) Prognostic value of exercise EKG testing in asymptomatic normotensive subjects. A prospective matched study. N Engl J Med 309: 1085-1089
71. Paillole C, Ruiz J, Juliard JM, Leblanc H, Gourgon R, Passa P (1995) Detection of coronary artery disease in diabetic patients. Diabetologia 38: 726-731
72. Bacci S, Villella M, Villella A et al. (2002) Screening for silent myocardial ischaemia in type 2 diabetic patients with additional atherogenic risk factors: applicability and accuracy of the exercise stress test. Eur J Endocrinol 147: 649-654
73. Janand-Delenne B, Savin B, Habib G, Bory M, Vague P, Lassmann-Vague V (1999) Silent myocardial ischemia in patients with diabetes: who to screen. Diabetes Care 22: 1396-1400
74. Cosson E, Paycha F, Paries J et al. (2004) Detecting silent coronary stenoses and stratifying cardiac risk in patients with diabetes: ECG stress test or exercise myocardial scintigraphy? Diabet Med 21: 342-348
75. Gerson MC, Khoury JC, Hertzberg VS, Fischer EE, Scott RC (1988) Prediction of coronary artery disease in a population of insulin-requiring diabetic patients: results of an 8-year follow-up study. Am Heart J 116: 820-826
76. Kim C, Kwok YS, Heagerty P, Redberg R (2001) Pharmacologic stress testing for coronary disease diagnosis: A meta-analysis. Am Heart J 142: 934-944
77. Bartunek J, Marwick TH, Rodrigues AC et al. (1996) Dobutamine-induced wall motion abnormalities: correlations with myocardial fractional flow reserve and quantitative coronary angiography. J Am Coll Cardiol 27: 1429-1436

78. Marwick TH, Nemec JJ, Pashkow FJ, Stewart WJ, Salcedo EE (1992) Accuracy and limitations of exercise echocardiography in a routine clinical setting. J Am Coll Cardiol 19: 74-81
79. Arruda-Olson AM, Juracan EM, Mahoney DW, McCully RB, Roger VL, Pellikka PA (2002) Prognostic value of exercise echocardiography in 5,798 patients: is there a gender difference? J Am Coll Cardiol 39: 625-631
80. McCully RB, Roger VL, Mahoney DW et al. (1998) Outcome after normal exercise echocardiography and predictors of subsequent cardiac events: follow-up of 1,325 patients. J Am Coll Cardiol 31: 144-149
81. Marwick TH, Case C, Short L, Thomas JD (2003) Prediction of mortality in patients without angina: use of an exercise score and exercise echocardiography. Eur Heart J 24: 1223-1230
82. Elhendy A, van Domburg RT, Poldermans D et al. (1998) Safety and feasibility of dobutamine-atropine stress echocardiography for the diagnosis of coronary artery disease in diabetic patients unable to perform an exercise stress test. Diabetes Care 21: 1797-1802
83. Hennessy TG, Codd MB, Kane G, McCarthy C, McCann HA, Sugrue DD (1997) Evaluation of patients with diabetes mellitus for coronary artery disease using dobutamine stress echocardiography. Coron Artery Dis 8: 171-174
84. Faglia E, Manuela M, Antonella Q et al. (2005) Risk reduction of cardiac events by screening of unknown asymptomatic coronary artery disease in subjects with type 2 diabetes mellitus at high cardiovascular risk: an open-label randomized pilot study. Am Heart J 149: e1-6
85. Iskandrian AS, Verani MS (1996) Exercise perfusion imaging in coronary artery disease: physiology and diagnosis. In: Nuclear cardiac imaging: principles and applications. Davis, Philadelphia, pp 73-143
86. Thomas GS, Miyamoto MI, Morello AP 3rd et al. (2004) Technetium 99m sestamibi myocardial perfusion imaging predicts clinical outcome in the community outpatient setting. The Nuclear Utility in the Community (NUC) Study. J Am Coll Cardiol 43: 213-223
87. Fleg JL, Gerstenblith G, Zonderman AB et al. (1990) Prevalence and prognostic significance of exercise-induced silent myocardial ischemia detected by thallium scintigraphy and electrocardiography in asymptomatic volunteers. Circulation 81: 428-436
88. Elhendy A, Schinkel A, Bax JJ, van Domburg RT, Poldermans D (2003) Long-term prognosis after a normal exercise stress Tc-99m sestamibi SPECT study. J Nucl Cardiol 10: 261-266
89. Kang X, Berman DS, Lewin H et al. (1999) Comparative ability of myocardial perfusion single-photon emission computed tomography to detect coronary artery disease in patients with and without diabetes mellitus. Am Heart J 137: 949-957
90. Rajagopalan N, Miller TD, Hodge DO, Frye RL, Gibbons RJ (2005) Identifying high-risk asymptomatic diabetic patients who are candidates for screening stress single-photon emission computed tomography imaging. J Am Coll Cardiol 45: 43-49

91. Miller TD, Rajagopalan N, Hodge DO, Frye RL, Gibbons RJ (2004) Yield of stress single-photon emission computed tomography in asymptomatic patients with diabetes. Am Heart J 147: 890-896
92. Wackers FJ, Young LH, Inzucchi SE et al. (2004) Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. Diabetes Care 27: 1954-1961
93. Sultan A, Piot C, Mariano-Goulart D et al. (2006) Myocardial perfusion imaging and cardiac events in a cohort of asymptomatic patients with diabetes living in southern France. Diabet Med 23: 410-418
94. Zellweger MJ, Hachamovitch R, Kang X et al. (2004) Prognostic relevance of symptoms versus objective evidence of coronary artery disease in diabetic patients. Eur Heart J 25: 543-550
95. Valensi P, Pariès J, Brulport-Cerisier V et al. (2005) Predictive value of silent myocardial ischemia for cardiac events in diabetic patients: influence of age in a French multicenter study. Diabetes Care 28: 2722-2727
96. Nitenberg A, Ledoux S, Valensi P, Sachs R, Attali JR, Antony I (2001) Impairment of coronary microvascular dilation in response to cold pressor-induced sympathetic stimulation in type 2 diabetic patients with abnormal stress thallium imaging. Diabetes 50: 1180-1185
97. O'Keefe JH Jr, Barnhart CS, Bateman TM (1995) Comparison of stress echocardiography and stress myocardial perfusion scintigraphy for diagnosing coronary artery disease and assessing its severity. Am J Cardiol 75: 25D-34D
98. Anand DV, Lim E, Hopkins D et al. (2006) Risk stratification in uncomplicated type 2 diabetes: prospective evaluation of the combined use of coronary artery calcium imaging and selective myocardial perfusion scintigraphy. Eur Heart J 27:713-721
99. Moreno PR, Murcia AM, Palacios IF et al. (2000) Coronary composition and macrophage infiltration in atherectomy specimens from patients with diabetes mellitus. Circulation 102: 2180-2184100. Detrano R, Guerci AD, Carr JJ et al. (2008) Coronary calcium as a predictor of coronary events in four racial or ethnic groups. N Engl J Med 27:1336-1345

CHAPTER 7

Usefulness of Carotid Intima Media Thickness in Patients with Diabetes Mellitus as a Predictor of Coronary Artery Disease

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ABSTRACT

Identification of asymptomatic patients with diabetes mellitus (DM) at increased risk for coronary artery disease (CAD) remains a challenge. Carotid intima media thickness (CIMT) has been proposed as a surrogate marker for CAD but only limited data are available. The purpose of the study was to evaluate the potential of CIMT for prediction of CAD in asymptomatic patients with DM. Prospectively, CIMT of the left and right common carotid artery was assessed by B-mode ultrasound in 150 asymptomatic diabetic patients (age 50±13 years, male 83). In addition, non-invasive multi-slice computed tomography (MSCT) coronary angiography was performed to relate CIMT to the presence and severity of CAD. For this purpose, patients were classified as having 1) no atherosclerosis, 2)non-obstructive atherosclerosis or 3)obstructive stenosis with ≥50% luminal diameter narrowing. Mean CIMT increased significantly from 0.58±0.08 mm in patients with normal coronary arteries (n=59, 39%) to 0.67±0.12 mm in patients with non-obstructive atherosclerosis (n=54, 36%). Highest mean CIMT (0.75±0.12 mm) was observed in patients with obstructive stenosis (n=36, 25%, P<0.01). ROC curve analysis yielded a sensitivity and specificity of respectively 85% and 72% with a CIMT cut-off value of 0.67 mm, for predicting obstructive coronary atherosclerosis. Multivariate analysis of baseline risk factors showed CIMT to be an independent predictor of any- and obstructive atherosclerosis (P<0.01). In conclusion, significant relation was shown between CIMT and the presence and severity of CAD in asymptomatic patients with DM. Assessment of CIMT may be useful to identify diabetic patients at higher risk for CAD.

INTRODUCTION

Presence of diabetes mellitus (DM) confers a substantially increased risk of coronary artery disease (CAD).[1] Moreover, in patients with DM, CAD often progresses without evident symptoms (silent ischemia). Therefore, current guidelines recommend stringent primary prevention with lipid and blood pressure goals similar to secondary prevention, in all patients with DM.[2] Nevertheless, within the asymptomatic diabetic population, some patients may be at relatively low risk and require limited medical therapy and risk modification, while others at elevated risk may benefit from more extensive cardiac evaluation and intensive medical therapy. Accordingly, a clear need exists for more individualized risk stratification. However, at present no consensus exists on the most appropriate approach for the identification of diabetic patients who may benefit from further non-invasive assessment of CAD. Possibly, initial assessment of cardiovascular risk may be performed by means of carotid intima media thickness (CIMT), which serves as a non-invasive marker of subclinical atherosclerosis.[3] Although this technique has been extensively studied in the general population [4-8], less data are available concerning its relation with CAD in asymptomatic patients with diabetes.[3,9] The current study was designed to prospectively evaluate the relation between CIMT and the presence and extent of CAD as assessed non-invasively by Multi-Slice Computed Tomography (MSCT) in asymptomatic diabetic patients, and thereby explore the potential role of CIMT as a tool for identification of diabetic individuals at higher risk for cardiovascular disease.

METHODS

One hundred fifty asymptomatic patients with DM were prospectively included and clinically referred for cardiovascular screening. Cardiovascular risk factors were derived through a structured interview, physical examination and laboratory analysis. Hypertension was defined as a blood pressure >140/90 mmHg or treatment with antihypertensive medication. Hypercholesterolemia was defined as a total cholesterol level >5.0 mmol/L or use of lipid lowering medication. All patients underwent assessment of the CIMT and non-invasive MSCT coronary angiography as part of their clinical evaluation. Exclusion criteria consisted of ventricular and supraventricular arrhythmia and contraindications for the use of iodinated contrast media.

Imaging was performed with a 64-slice MSCT scanner (Aquilion64, Toshiba Medical Systems, Japan). If necessary and tolerated, oral beta-blockers (metoprolol 50 mg or 100 mg) were provided 1 hour preceding the scan to achieve a heart rate <65 beats per minute. Initially, a non-enhanced prospective electrocardiographically gated scan, triggered at 75% of the R-R interval with 4 x 3.0 mm collimation was obtained to measure coronary artery calcium (CAC) score and determine the start and end position of the helical scan.

Thereafter, MSCT angiography was performed using the following parameters: collimation 64 x 0.5 mm, tube rotation time 400, 450 or 500 ms depending on the heart rate, tube current 300 or 350 mA, tube voltage 120 kV. Non-ionic contrast material was administered in the antecubital vein at a flow rate of 5 ml/L and the amount of 90–105 ml (depending on the total scan time), followed by 50 ml of saline solution flush. Automated bolus-tracking in the aortic root was used for the timing of the scan. Images were acquired with simultaneous ECG registration during a single breath hold of approximately 10 seconds. Segmental reconstruction algorithm was applied to generate a single image from the data of one, two or three consecutive heartbeats. Images were reconstructed in the cardiac phase showing least motion artifacts. In general, the end-diastolic phase was used. However, additional reconstructions were made throughout the entire cardiac cycle if necessary to improve image quality. Subsequently, the images were transferred to a remote workstation (Vitrea 2, Vital Images, Minnetonka, USA) for post-processing.

All data were evaluated with a remote workstation using dedicated software (Vitrea2, Vital Images, Minnetonka, USA). In each patient, coronary calcium was identified as a dense area in the coronary artery exceeding the threshold of 130 Hounsfield units. The total Agatston score was determined for each patient. Patients with a CAC score >100 were classified as having increased CAC.

MSCT coronary angiography images were interpreted by two experienced observers blinded to the patient characteristics. Discrepancies in interpretation were resolved by consensus. The presence of coronary atherosclerosis was visually evaluated on axial images and curved multiplanar reconstructions in at least two orthogonal planes. Obstructive coronary atherosclerosis was defined as the presence of luminal narrowing ≥ 50%.

Carotid arteries were evaluated using high resolution B-mode ultrasound with a 10-MHz linear transducer (Art.Lab Esaote Picus, Genova, Italy). The ultrasound device was connected to an acquisition modem with an automatic boundary detection system (Art. Lab Esaote Picus, Genova, Italy). The CIMT was thereby quantified semi-automatically. All measurements were performed by the same two trained ultrasonographers who were blinded to all clinical information.

With the patient in supine position, measurements of the CIMT were performed throughout 10 mm segments across the far wall of the left and right common carotid artery (CCA), at a point most proximal to the carotid bifurcation. The probe was moved to obtain measurements of the CCA at four angles on the right (180°, 150°, 120° and 90°) and at four angles on the left (180°, 210°, 240° and 270°). For each measured segment the mean and maximum CIMT value was acquired automatically throughout the 10 mm vessel

length. Finally, the average of the mean CIMT values of all segments was calculated to determine mean CIMT per patient.

Of note, all measurements complied with the consensus recommendations that were recently proposed to standardize CIMT methodology and to restrict variations in definition of CIMT (mean or maximum IMT), location of measurements and the technique used (manual or semi-automatic edge detecting systems).[10-12]

Continuous variables were expressed as means ± standard deviation. Categorical variables were expressed as numbers (percentages). Firstly, the relationship between CAC score and CIMT was analyzed. For this purpose the study population was divided into patients with a CAC score 0-100 and those with an elevated CAC score study defined as a CAC score >100. Average CIMT and standard deviation were calculated in each group. The independent T-test was used to assess the difference in mean CIMT between the 2 groups. To evaluate the relation between coronary atherosclerosis and CIMT, the study population was categorized according to the results of the MSCT coronary angiography. MSCT coronary angiograms were stratified as normal in absence of atherosclerosis. In presence of coronary atherosclerosis, the examinations were further sub-classified as non-obstructive (defined as coronary narrowing <50%) or obstructive atherosclerosis (defined as coronary narrowing ≥50%). Average CIMT and standard deviation were calculated for all three groups, and the independent T-test was applied to evaluate the difference in mean CIMT. Using ROC curve analysis cut-off values were chosen for mean CIMT. Subsequently, sensitivity and specificity values were calculated for predicting: 1) CAC score >100, 2) presence of any coronary atherosclerosis and 3) presence of obstructive coronary atherosclerosis. To determine the relationship between cardiovascular risk factors and coronary atherosclerosis, a univariate analysis of baseline clinical characteristics was performed to identify the potential predictors of any coronary atherosclerosis on MSCT coronary angiography. Thereafter, risk factors with a P value <0.05 were included in a multiple regression analysis to identify the independent predictors of any coronary atherosclerosis. A similar procedure was repeated to identify the predictors of obstructive coronary atherosclerosis on MSCT coronary angiography. Finally, the incremental value of CIMT over other clinical predictors of obstructive coronary atherosclerosis was assessed by calculating the global chi-square test. Statistical analyses were performed using SPSS software (version 12.0.1, Inc., Chicago, Illinois). P values <0.05 were considered statistically significant.

RESULTS

The baseline characteristics of the study population are provided in Table1.

Mean CAC score was 211 ± 448 in the total population. In 102 patients (68%) CAC was absent or ≤100, while 48 patients (32%) had a CAC score >100. MSCT coronary angiography showed normal coronaries in 59 patients (39%). Coronary atherosclerosis was present in 91 patients (61%). Within this group, 54 patients (36% of total population) had non-obstructive atherosclerosis defined as luminal narrowing <50%, and 37 patients (25% of total population) were observed with obstructive atherosclerosis (luminal narrowing ≥50%).

CIMT ranged from 0.43 to 1.01 mm, with an average of 0.65 ± 0.12 mm. Mean CIMT was higher in patients with a CAC score >100 (0.74 ± 0.13 mm) as compared to those with a CAC score in the range 0-100 (0.61 ± 0.10 mm) (P <0.001) (Figure 1A). Using ROC curve analysis a cut-off value of 0.65 mm was identified for CIMT. This cut-off value yielded a sensitivity and a specificity of respectively 72% and 70% for predicting a CAC score >100 (Figure 1B).

After stratification according to MSCT angiography results, lowest mean CIMT was observed in patients with normal coronaries (0.58 ± 0.08 mm). Mean CIMT increased to an average of 0.67 ± 0.12 mm in patients with non-obstructive coronary atherosclerosis (P <0.001) and further to an average of 0.75 ± 0.12 mm in patients stratified as having obstructive coronary atherosclerosis (P = 0.002) (Figure 2A). ROC curve analysis resulted in a sensitivity and specificity of 76% and 71% with a cut-off value of 0.62 mm for predicting any coronary atherosclerosis (Figure 2B). Similarly, ROC curve analysis resulted in a sensitivity and specificity of 85% and 72% with a cut-off value of 0.67 mm for predicting obstructive coronary atherosclerosis (Figure 2C).

Results of the univariate – and multivariate regression analysis of the baseline characteristics for prediction of any coronary atherosclerosis and obstructive coronary atherosclerosis are provided in Table 2. CIMT, DM duration and family history of CAD were identified as independent predictors of any coronary atherosclerosis. Likewise, age, plasma HbA1c and CIMT were identified as independent predictors of obstructive coronary atherosclerosis. Importantly, calculation of global chi-square showed significant incremental value of CIMT values above age and plasma HbA1c in the prediction of obstructive coronary atherosclerosis (Figure 3).

Table 1. Characteristics of the study population (*n* = 150)

Variable	
Age (years)	50 ± 13
Men	83 (55%)
Body mass index (Kg/m2)	28 ± 6
Smokers	38 (25%)
Hypercholesterolemia*	101 (68%)
Hypertension†	87 (58%)
Diabetes Mellitus duration (years)	14 ± 13
Glycosylated hemoglobin A1c (mmol/L)	8.1 ± 1.7

Data are averages ± standard deviation or number of patients (%).

* Total cholesterol level > 5.0 mmol/L or use of cholesterol-lowering medication.

† Blood pressure > 140 mmHg or treatment with antihypertensive medication.

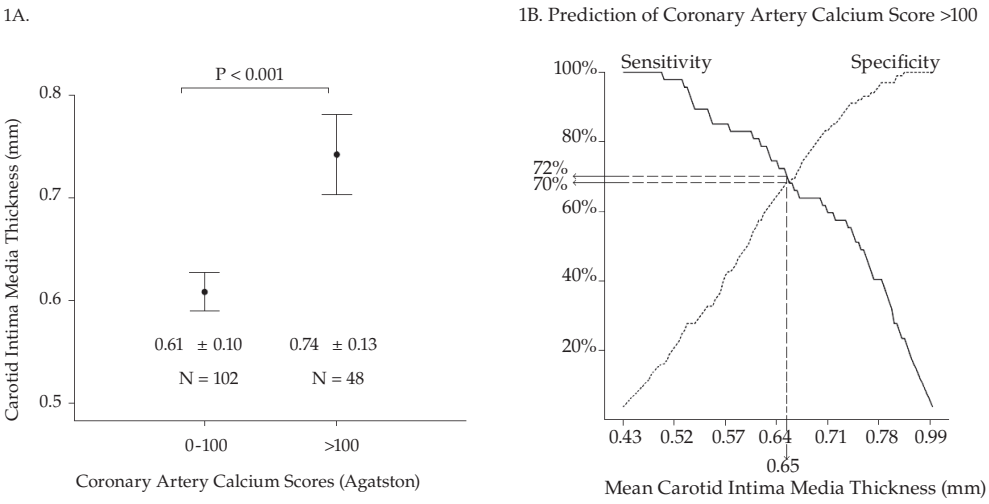

Figure 1. Relation between CIMT and CAC scores. (*A*) Mean CIMT was significantly higher in patients with a CAC score >100. (*B*) Receiver operating characteristics curve analysis yielded a sensitivity and specificity of 72% and 70% with a CIMT cut-off value of 0.65 mm for prediction of a CAC score >100.

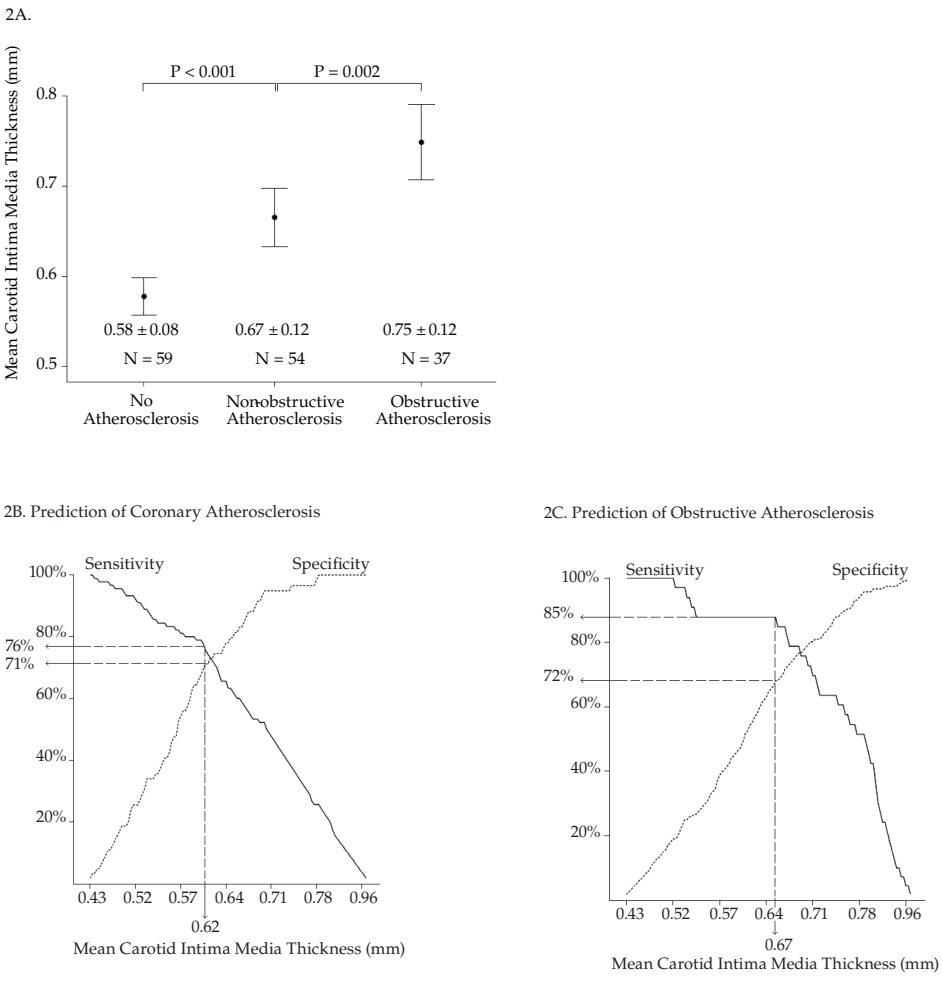

Figure 2. Relation between CIMT and coronary atherosclerosis. (*A*) Mean CIMT increased significantly with degree of coronary atherosclerosis. Receiver operating characteristics curve curve analysis yielded (*B*) a sensitivity and specificity of 76% and 71% with a CIMT cut-off value of 0.62 mm for prediction of any coronary atherosclerosis, and (*C*) a sensitivity and specificity of 85% and 72% with a CIMT cut-off value of 0.67 mm for prediction of obstructive coronary atherosclerosis.

Table 2. Predictors of coronary atherosclerosis and obstructive coronary atherosclerosis on Multi-Slice Computed Tomography angiography.

	Atherosclerosis				Obstructive Atherosclerosis			
Variable	*Univariate*		*Multivariate*		*Univariate*		*Multivariate*	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
CIMT*	1.01 (1.01-1.02)	<0.001	1.01 (1.00-1.01)	0.003	1.01 (1.01-1.02)	<0.001	1.01 (1.00-1.01)	0.002
Age*	1.11 (1.07-1.15)	<0.001	1.05 (1.00-1.01)	0.05	1.10 (1.06-1.15)	<0.001	1.05 (1.00-1.11)	0.07
Male Gender	1.10 (0.57-2.14)	0.77	-	-	1.52 (0.69-3.38)	0.30	-	-
Duration of DM*	1.04 (1.01-1.07)	0.02	1.05 (1.01-1.09)	0.01	0.99 (0.96-1.03)	0.76	-	-
Glycosylated hemoglobin A1c*	1.20 (0.98-1.47)	0.07	-	-	1.39 (1.11-1.73)	0.004	1.50 (1.13-2.00)	0.01
Hypertension	3.85 (1.93-7.71)	<0.001	1.45 (0.59-3.55)	0.41	4.20 (1.61-10.93)	0.003	1.94 (0.63-6.05)	0.25
Hypercholesterolemia	2.80 (1.37-5.75)	0.01	2.31 (0.92-5.84)	0.08	1.06 (0.46-2.46)	0.89	-	-
Body mass index*	0.99 (0.94-1.05)	0.84	-	-	0.98 (0.92-1.05)	0.65	-	-
Smoker	1.01 (0.47-2.14)	0.99	-	-	1.37 (0.58-3.22)	0.47	-	-

* Ratio per unit increase.

CI = confidence interval.

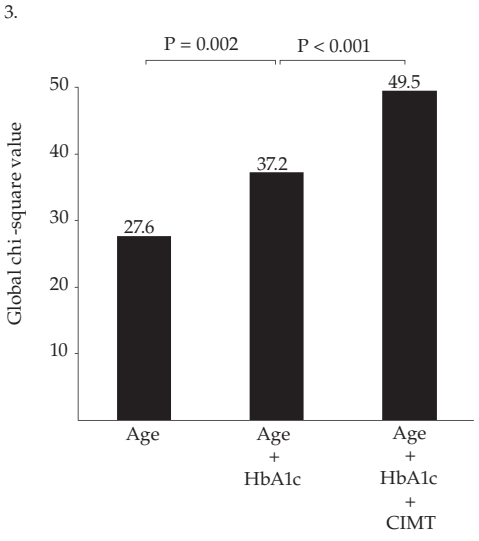

Figure 3. Incremental value of CIMT over significant clinical predictors of obstructive coronary atherosclerosis. Addition of CIMT value, to age and HbA1c, resulted in a significant increase of global chi-square from 37.7 to 49.5 (P <0.001).

DISCUSSION

In this asymptomatic population recruited from a diabetes clinic, a significant relation was observed between CIMT and the presence of atherosclerosis determined by CAC and MSCT coronary angiography. CIMT increased with elevated CAC scores as well as with the degree of coronary atherosclerosis on MSCT coronary angiography. In addition, ROC curve analysis of the CIMT relation with variables of atherosclerosis on MSCT resulted in reasonable sensitivity values for prediction of CAD. Good sensitivity was observed for detection of obstructive CAD in particular. Furthermore, after correction for traditional risk factors CIMT was found to be an independent predictor of any- and obstructive coronary atherosclerosis.

DM is considered a CAD equivalent and currently rigorous primary prevention is recommended by AHA/ADA in all diabetic patients.[2] However, whether all diabetic patients equally benefit from this treatment strategy remains uncertain. A risk stratification algorithm allowing exclusion of patients at lower risk from further evaluation and intensive treatment would improve cost-effectiveness, but remains a challenge. Thus far, various strategies have been recommended for individual risk stratification within the diabetic population. Primarily, the AHA/ADA 1998 guidelines suggested more aggressive medical treatment and assessment of CAD only in presence

of two additional risk factors.[13] However, the Detection of Ischemia in Asymptomatic Diabetics (DIAD) study based on 1123 asymptomatic diabetic patients showed the number of cardiovascular risk factors to be a poor predictor of hemodynamically relevant CAD.[14] Moreover, that particular study revealed that a selection strategy based on a minimum of two additional risk factors underestimates the presence of CAD in a large proportion of patients, as 41% of patients with myocardial ischemia would not have been identified. The clinical utility of more refined risk prediction models may also be limited as the Framingham score has been shown to underestimate event rates in DM as compared to the non diabetic population.[15] The SCORE and DECODE models incorporate DM in a categorical fashion and have been criticized of inadequate risk estimation due to negligence of glycemic level.[15] Prospective evaluation of the diabetes specific UKPDS score in an asymptomatic diabetic population suggested less underestimation of coronary events as compared to the Framingham score.[16] Nevertheless, also the UKPDS score showed a poor relation between actual and predicted coronary events on an individual basis. It is assumable that direct estimation of atherosclerosis as can be obtained with CIMT may provide more accurate risk stratification.

Before CIMT can be advocated as a potential cardiovascular screening tool in diabetic patients asymptomatic for CAD, it is necessary to evaluate the association between CIMT measurement and the presence and extent of CAD in this population. Previous histological analyses have illustrated a strong agreement between the ultrasonographic estimation and actual IMT values.[17] Evaluation of IMT provides a direct measure of the presence of atherosclerosis, which is a systemic disease.[17] In the general population, association of CIMT with the presence of current CAD, as well as its significant predictive value for cardiac events have been shown.[4-8] In diabetic patients, the relation between CIMT and CAD has been assessed in sub-analyses of the ARIC and the IRAS investigations to reveal higher mean CIMT in patients with established CAD.[18,19] However, the association between CIMT and CAD has not been examined in asymptomatic patients with DM. In the current study, therefore, we evaluated in asymptomatic diabetic patients the relation between CIMT and CAD, as assessed by MSCT, a technique which not only allows assessment of CAC but also provides information on coronary integrity and degree of coronary atherosclerosis.[20] We observed a strong relation between CIMT and CAC scores as well as with the degree of coronary atherosclerosis. Importantly, CIMT was shown to be an independent predictor of coronary atherosclerosis in asymptomatic diabetic patients after correction for baseline risk factors. Furthermore, CIMT showed good sensitivity for the prediction of obstructive CAD, and was revealed to have incremental value over clinical risk factors in the prediction of obstructive CAD.

Based on these findings, CIMT appears to be a useful tool to identify asymptomatic diabetic patients at higher risk for CAD. Two previous prognostic studies in asymptomatic

diabetic patients, which showed CIMT to be predictive of future cardiovascular events, further support this concept.[21,22] Although direct assessment of coronary atherosclerosis by means of CAC or MSCT coronary angiography may provide more detailed information and allow more accurate risk stratification [23], these techniques involve radiation and higher costs. In contrast, ultrasonographic measurement of CIMT offers the advantage of being truly non-invasive, inexpensive and radiation-free. Accordingly, CIMT may be particularly useful in asymptomatic diabetic patients as an initial step to select patients requiring more aggressive medical therapy and further cardiovascular evaluation.

Several limitations must be acknowledged. The prognostic value of CIMT was not evaluated as no follow-up data were available. Furthermore, the current analysis was restricted to the evaluation of the relation between CIMT and CAD in diabetic patients, and assessment did not include a non-diabetic control group. As MSCT coronary angiography is accompanied with radiation exposure, it is not feasible to perform a similar assessment in asymptomatic subjects free of cardiovascular risk.

REFERENCES

1. Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham study. *JAMA* 1979; 241:2035-2038.
2. Buse JB, Ginsberg HN, Bakris GL, Clark NG, Costa F, Eckel R, Fonseca V, Gerstein HC, Grundy S, Nesto RW, Pignone MP, Plutzky J, Porte D, Redberg R, Stitzel KF, Stone NJ; American Heart Association; American Diabetes Association. Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. *Circulation* 2007; 115:114-126.
3. Raggi P, Bellasi A, Ratti C. Ischemia imaging and plaque imaging in diabetes: complementary tools to improve cardiovascular risk management. *Diabetes Care* 2005; 28: 2787-2794.
4. Burke GL, Evans GW, Riley WA, Sharrett AR, Howard G, Barnes RW, Rosamond W, Crow RS, Rautaharju PM, Heiss G. Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study. *Stroke* 1995; 26:386-391.
5. Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, Clegg LX. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987-1993. *Am J Epidemiol* 1997; 146:483-494.
6. Iglesias del Sol A, Bots ML, Grobbee DE, Hofman A, Witteman JC. Carotid intima-media thickness at different sites: relation to incident myocardial infarction; The Rotterdam Study. *Eur Heart J* 2002; 23:934-940.
7. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med* 1999; 340:14-22.
8. Lorenz MW, von Kegler S, Steinmetz H, Markus HS, Sitzer M. Carotid intima-media thickening indicates a higher vascular risk across a wide age range: prospective data from the Carotid Atherosclerosis Progression Study (CAPS). *Stroke* 2006; 37:87-92.
9. Redberg RF, Greenland P, Fuster V, Pyörälä K, Blair SN, Folsom AR, Newman AB, O'Leary DH, Orchard TJ, Psaty B, Schwartz JS, Starke R, Wilson PW. Prevention Conference VI: Diabetes and Cardiovascular Disease: Writing Group III: risk assessment in persons with diabetes. *Circulation* 2000; 105:e144-e152.
10. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, Najjar SS, Rembold CM, Post WS; American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *J Am Soc Echocardiogr* 2008; 21:93-111.

11. Greenland P, Abrams J, Aurigemma GP, Bond MG, Clark LT, Criqui MH, Crouse JR 3rd, Friedman L, Fuster V, Herrington DM, Kuller LH, Ridker PM, Roberts WC, Stanford W, Stone N, Swan HJ, Taubert KA, Wexler L. Prevention Conference V: Beyond secondary prevention: identifying the high-risk patient for primary prevention: noninvasive tests of atherosclerotic burden: Writing Group III. *Circulation* 2000; 101:E16-E22.
12. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, Csiba L, Desvarieux M, Ebrahim S, Fatar M, Hernandez Hernandez R, Jaff M, Kownator S, Prati P, Rundek T, Sitzer M, Schminke U, Tardif JC, Taylor A, Vicaut E, Woo KS, Zannad F, Zureik M. Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. *Cerebrovasc Dis* 2007; 23:75-80.
13. Consensus development conference on the diagnosis of coronary heart disease in people with diabetes. *Diabetes Care* 1998; 21:1551-1559.
14. Wackers FJ, Young LH, Inzucchi SE, Chyun DA, Davey JA, Barrett EJ, Taillefer R, Wittlin SD, Heller GV, Filipchuk N, Engel S, Ratner RE, Iskandrian AE; Detection of Ischemia in Asymptomatic Diabetics Investigators. Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. *Diabetes Care* 2004; 27:1954-1961.
15. Coleman RL, Stevens RJ, Retnakaran R, Holman RR. Framingham, SCORE, and DECODE risk equations do not provide reliable cardiovascular risk estimates in type 2 diabetes. *Diabetes Care* 2007; 30:1292-1293.
16. Guzder RN, Gatling W, Mullee MA, Mehta RL, Byrne CD. Prognostic value of the Framingham cardiovascular risk equation and the UKPDS risk engine for coronary heart disease in newly diagnosed Type 2 diabetes: results from a United Kingdom study. *Diabet Med* 2005; 22:554-562.
17. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation* 1986; 74:1399-1406.
18. Haffner SM, Agostino RD Jr, Saad MF, O'Leary DH, Savage PJ, Rewers M, Selby J, Bergman RN, Mykkänen L. Carotid artery atherosclerosis in type-2 diabetic and nondiabetic subjects with and without symptomatic coronary artery disease (The Insulin Resistance Atherosclerosis Study). *Am J Cardiol* 2000; 85:1395-1400.
19. Lee CD, Folsom AR, Pankow JS, Brancati FL; Atherosclerosis Risk in Communities (ARIC) Study Investigators. Cardiovascular events in diabetic and nondiabetic adults with or without history of myocardial infarction. *Circulation* 2004; 109:855-860.

20. Van Mieghem CA, McFadden EP, de Feyter PJ, Bruining N, Schaar JA, Mollet NR, Cademartiri F, Goedhart D, de Winter S, Granillo GR, Valgimigli M, Mastik F, van der Steen AF, van der Giessen WJ, Sianos G, Backx B, Morel MA, van Es GA, Zalewski A, Serruys PW. Noninvasive detection of subclinical coronary atherosclerosis coupled with assessment of changes in plaque characteristics using novel invasive imaging modalities: the Integrated Biomarker and Imaging Study (IBIS). *J Am Coll Cardiol* 2006; 47:1134-1142.
21. Yamasaki Y, Kodama M, Nishizawa H, Sakamoto K, Matsuhisa M, Kajimoto Y, Kosugi K, Shimizu Y, Kawamori R, Hori M. Carotid intima-media thickness in Japanese type 2 diabetic subjects: predictors of progression and relationship with incident coronary heart disease. *Diabetes Care* 2000; 23:1310-1315.
22. Bernard S, Sérusclat A, Targe F, Charrière S, Roth O, Beaune J, Berthezène F, Moulin P. Incremental predictive value of carotid ultrasonography in the assessment of coronary risk in a cohort of asymptomatic type 2 diabetic subjects. *Diabetes Care* 2005; 28:1158-1162.
23. Folsom AR, Kronmal RA, Detrano RC, O'Leary DH, Bild DE, Bluemke DA, Budoff MJ, Liu K, Shea S, Szklo M, Tracy RP, Watson KE, Burke GL. Coronary artery calcification compared with carotid intima-media thickness in the prediction of cardiovascular disease incidence: the Multi-Ethnic Study of Atherosclerosis (MESA). *Arch Intern Med* 2008; 168:1333-1339.

CHAPTER 8

Increased Carotid Intima Media Thickness as a Predictor of the Presence and Extent of Abnormal Myocardial Perfusion in Type 2 Diabetes

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ABSTRACT

OBJECTIVE

Identification of asymptomatic patients with type 2 diabetes at increased risk for coronary artery disease remains a challenge. We evaluated the potential of carotid intima media thickness (CIMT) for prediction of abnormal myocardial perfusion in this population.

RESEARCH DESIGN AND METHODS

CIMT and SPECT myocardial perfusion imaging were assessed in 98 asymptomatic patients with type 2 diabetes. An increased CIMT was defined as ≥75th percentile of reference values.

RESULTS

Increased CIMT was an independent predictor of the extent of abnormal perfusion (P <0.001). In patients with increased CIMT as compared to patients with normal CIMT, abnormal perfusion (75% vs. 9%) and severely abnormal perfusion (28% vs. 3%) were observed more frequently.

CONCLUSIONS

Increased CIMT was significantly related to the presence and extent of abnormal myocardial perfusion. Assessment of CIMT may be useful to identify asymptomatic patients with type 2 diabetes at higher risk for coronary artery disease.

INTRODUCTION

Identification of asymptomatic patients with type 2 diabetes at increased risk for coronary artery disease (CAD) remains a challenge. In the current study, we evaluated the potential of carotid intima media thickness (CIMT) to identify asymptomatic patients with type 2 diabetes at higher risk for abnormal myocardial perfusion.

RESEARCH DESIGN AND METHODS

Prospectively, 98 consecutive asymptomatic patients with type 2 diabetes (1), were recruited from a routine outpatient diabetes clinic, and referred for cardiovascular risk-stratification. Asymptomatic status was confirmed using the Rose questionnaire (2). All patients underwent myocardial perfusion imaging by SPECT and CIMT assessment.

SPECT data acquisition and data analysis

Myocardial perfusion imaging was performed using ECG-gated SPECT with ^{99m}Tc-sestamibi, during pharmacological stress and rest, according to protocols described previously (3).

Using a 17-segment model tracer uptake in each segment was evaluated by two observers in consensus, by use of a 5-point scoring system (4). The total segmental score during stress was used to determine the extent of abnormal perfusion as reflected by the summed stress score (SSS). Abnormal perfusion was defined as SSS ≥3, and severely abnormal perfusion as SSS ≥8.

CIMT measurement and data analysis

CIMT was assessed using high resolution B-mode ultrasound with a 10-MHz linear transducer, with an automatic boundary detection system (Art.Lab-Esaote-Picus, Genova, Italy). Measurements were performed by an experienced sonographer blinded to clinical information (5). Mean CIMT was assessed throughout 10-mm segments, at four angles, across the far wall of the right and left common carotid artery (CCA). The average of the mean CIMT values of the 4 segments was calculated to determine the mean right and left CIMT per patient.

CIMT values ≥75th percentile (per age and gender category) are defined as increased, indicating elevated cardiovascular risk (5). In the current study, the mean CIMT was compared with reference values from the Multi-Ethnic Study of Atherosclerosis (5). Patients were thereby stratified as having normal CIMT (CIMT <75th percentile), or increased CIMT (CIMT ≥75th percentile in at least one CCA).

Statistical analysis

First, average SSS and standard deviations were calculated in patients with normal or increased CIMT. The independent T-test was used to assess the difference in mean SSS between the two groups.

Thereafter, univariate analysis of baseline characteristics including age, positive family history of CAD, smoking, hypertension, hypercholesterolemia, body mass index, nephropathy (urine albumin/creatinine ≥3.5 mg/mmol), fasting glucose, glycated-hemoglobin (by chromatography) (6), retinopathy, peripheral arterial disease and increased CIMT, was performed to identify potential predictors of the extent of abnormal perfusion (SSS). Subsequently, risk factors with a *P* value <0.05 were included in a linear multiple regression model to identify independent predictors of SSS.

Finally, the prevalence of abnormal perfusion (SSS≥3) and severely abnormal perfusion (SSS≥8) was compared between patients with normal and increased CIMT.

RESULTS

Briefly, the mean age of the study population was 54 ± 11 years with the majority of patients being male (*n* = 50, 51%).

Mean SSS was 3.1 ± 4.2 in the total population. Overall, 34 patients (35%) showed abnormal perfusion (SSS ≥3), including severely abnormal perfusion (SSS ≥8) in 14 patients (14%).

Average CIMT was 0.68 ± 0.12 mm. Comparison with reference values revealed normal CIMT in 60 patients (61%), while in the remaining 38 patients (39%) an increased CIMT value was observed in at least one CCA.

CIMT versus extent of abnormal perfusion

The mean SSS increased significantly from 1.2±2.1 in patients with normal CIMT, to 5.6±4.6 in patients with increased CIMT (P<0.001).

Age, smoking, hypertension, nephropathy and increased CIMT, were identified as potential predictors of SSS on SPECT, in a univariate regression model. Importantly, after adjustment for age, smoking, hypertension and nephropathy in a multivariate model, increased CIMT remained a significant predictor of SSS (*P* <0.001) (ß = 4.41 [95% CI 3.05-5.76]).

CIMT versus prevalence of abnormal perfusion

Abnormal perfusion was present in 9% of patients with normal CIMT versus 75% of patients with increased CIMT (Figure 1*A*). Notably, prevalence of severely abnormal perfusion increased from 3% in patients with normal CIMT to 28% in those with increased CIMT (Figure 1*B*).

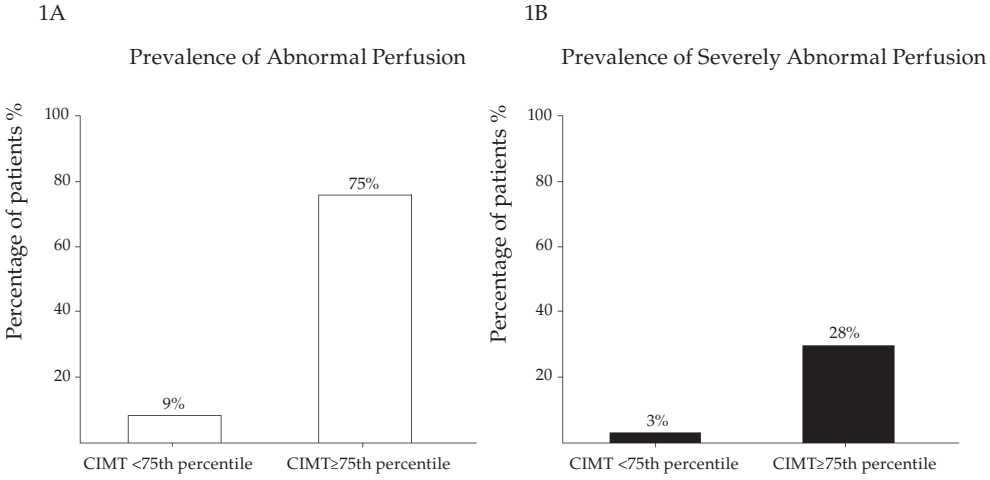

Figure 1. Relation between CIMT and myocardial perfusion imaging. Prevalence of abnormal perfusion (Figure 1*A*), and severely abnormal perfusion (Figure 1*B*), was higher in patients with increased CIMT.

CONCLUSIONS

The prognostic value of SPECT imaging has been confirmed in diabetic patients (7). In particular, a favorable cardiovascular prognosis has been described in patients with normal myocardial perfusion, whereas significantly higher adverse event rates were observed in patients with severely abnormal perfusion (8). SPECT has therefore been proposed as a screening tool for identification of asymptomatic diabetic patients with obstructive CAD (9). However, considering the high global prevalence of type 2 diabetes, a broad screening strategy of all asymptomatic patients using SPECT perfusion imaging does not appear feasible or cost-effective (10). AHA/ADA therefore initially suggested more aggressive medical treatment and assessment of CAD only in the presence of two additional risk factors (9). Nonetheless, baseline analysis of SPECT data in the DIAD study demonstrated that a selection strategy based on a minimum of two additional risk factors underestimates the presence of abnormal perfusion in a large proportion of patients (41%)(11). Accordingly, the key question remains how asymptomatic diabetic patients with severe CAD should be identified from the general diabetic population.

Assessment of CIMT has been previously proposed for this purpose (12). Moreover, the truly non-invasive, inexpensive and radiation-free nature of CIMT may represent an important advantage over other suggested screening techniques such as coronary calcium

scoring (5). However, the relation of CIMT with CAD has not been fully established in asymptomatic diabetic patients. In the current study, increased CIMT was shown to be a strong predictor of the extent of abnormal perfusion and improved identification of patients with severely abnormal perfusion (28%). Normal CIMT values on the other hand were associated with a low risk for abnormal perfusion. Importantly, only few asymptomatic diabetic patients with normal CIMT values had severely abnormal perfusion (3%). However, it must be acknowledged that a non-diabetic control group was not available for comparison. Also, patients were referred from a diabetes clinic and may thus represent a more high-risk group than the general asymptomatic population with diabetes. Nevertheless our findings suggest that initial risk-stratification using CIMT may allow selective referral of asymptomatic patients with type 2 diabetes requiring further imaging and intensification of therapy; thereby improving patient outcome while maintaining cost-effectiveness.

REFERENCES

1. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183, 1997
2. Rose G, McCartney P, Reid DD: Self-administration of a questionnaire on chest pain and intermittent claudication. *Br J Prev Soc Med* 31:42-48, 1977
3. Scholte AJ, Schuijf JD, Kharagjitsingh AV, Dibbets-Schneider P, Stokkel MP, van der Wall EE, Bax JJ: Prevalence and predictors of an abnormal stress myocardial perfusion study in asymptomatic patients with type 2 diabetes mellitus. *Eur J Nucl Med Mol Imaging* 36:567-575, 2009
4. Hansen CL, Goldstein RA, Akinboboye OO, Berman DS, Botvinick EH, Churchwell KB, Cooke CD, Corbett JR, Cullom SJ, Dahlberg ST, Druz RS, Ficaro EP, Galt JR, Garg RK, Germano G, Heller GV, Henzlova MJ, Hyun MC, Johnson LL, Mann A, McCallister BD Jr, Quaife RA, Ruddy TD, Sundaram SN, Taillefer R, Ward RP, Mahmarian JJ; American Society of Nuclear Cardiology: Myocardial perfusion and function: single photon emission computed tomography. *J Nucl Cardiol* 14:e39-60, 2007
5. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, Najjar SS, Rembold CM, Post WS; American Society of Echocardiography Carotid Intima-Media Thickness Task Force: Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *J Am Soc Echocardiogr* 21:93-111, 2008
6. Jeppsson JO, Kobold U, Barr J, Finke A, Hoelzel W, Hoshino T, Miedema K, Mosca A, Mauri P, Paroni R, Thienpont L, Umemoto M, Weykamp C; International Federation of Clinical Chemistry and Laboratory Medicine (IFCC): Approved IFCC reference method for the measurement of HbA1c in human blood. *Clin Chem Lab Med* 40:78-89, 2002
7. Valensi P, Pariès J, Brulport-Cerisier V, Torremocha F, Sachs RN, Vanzetto G, Cosson E, Lormeau B, Attali JR, Maréchaud R, Estour B, Halimi S: Predictive value of silent myocardial ischemia for cardiac events in diabetic patients: influence of age in a French multicenter study. *Diabetes Care* 2:2722-2727, 2005
8. Kang X, Berman DS, Lewin HC, Cohen I, Friedman JD, Germano G, Hachamovitch R, Shaw LJ: Incremental prognostic value of myocardial perfusion single photon emission computed tomography in patients with diabetes mellitus. *Am Heart J* 138:1025-1032, 1999
9. Consensus development conference on the diagnosis of coronary heart disease in people with diabetes: 10-11 February 1998, Miami, Florida. American Diabetes Association. *Diabetes Care* 21:1551-1559, 1998

10. Young LH, Wackers FJ, Chyun DA, Davey JA, Barrett EJ, Taillefer R, Heller GV, Iskandrian AE, Wittlin SD, Filipchuk N, Ratner RE, Inzucchi SE; DIAD Investigators: Cardiac outcomes after screening for asymptomatic coronary artery disease in patients with type 2 diabetes: the DIAD study: a randomized controlled trial. *JAMA* 301:1547-1555, 2009
11. Wackers FJ, Young LH, Inzucchi SE, Chyun DA, Davey JA, Barrett EJ, Taillefer R, Wittlin SD, Heller GV, Filipchuk N, Engel S, Ratner RE, Iskandrian AE; Detection of Ischemia in Asymptomatic Diabetics Investigators: Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. *Diabetes Care* 27:1954-1961, 2004
12. Raggi P, Bellasi A, Ratti C: Ischemia imaging and plaque imaging in diabetes: complementary tools to improve cardiovascular risk management. *Diabetes Care* 28: 2787-2794, 2005

CHAPTER 9

Relationship between Vascular Stiffness and Stress Myocardial Perfusion Imaging in Asymptomatic Patients with Diabetes

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ABSTRACT

Purpose

Vascular stiffness may potentially be used as a screening tool to identify asymptomatic patients with diabetes with abnormal myocardial perfusion. The purpose of this study was therefore to determine the association between vascular stiffness measured with pulse wave velocity (PWV) and augmentation index (AIx), and abnormal myocardial perfusion imaging (MPI) in asymptomatic patients with diabetes.

Methods

Prospectively, 160 asymptomatic patients with diabetes (mean age 51 yrs, male 87) underwent MPI with adenosine stress. Summed stress score (SSS) was determined per patient according to a 17 segment and 5 point score. Abnormal MPI (SSS ≥3) was sub-classified as moderate (SSS 3-7) or severe (SSS ≥8) MPI defects. Using applanation tonometry, the carotid-femoral PWV and the radial AIx corrected to 75 beats per minute were determined non-invasively.

Results

MPI was abnormal in 61 patients (38%), with severe MPI defects in 22 patients (14%). Mean PWV increased with deteriorating MPI from 8.4 ± 2.2 m/s in normal MPI to 9.0 ± 2.2 m/s in moderate MPI defects ($P = 0.11$), and to 11.1 ± 2.5 m/s in severe MPI defects ($P < 0.01$). Likewise, mean AIx increased from 18.4 ± 13.4% to 19.4 ± 10.7% ($P = 0.66$) and to 25.4 ± 9.0% ($P = 0.03$). After adjustment for age and other risk factors, PWV remained a significant predictor of severe MPI defects ($P = 0.01$, OR 1.50, 95% CI 1.11-2.00), whereas AIx was no longer significant ($P = 0.20$).

Conclusions

Vascular stiffness measured by PWV is associated with severe MPI defects in asymptomatic patients with diabetes.

INTRODUCTION

It is considered that the global prevalence of diabetes will approximately double in the next two decades [1]. Diabetes is associated with a marked increase in the incidence of cardiovascular morbidity and mortality, mainly attributable to coronary artery disease (CAD). Moreover, the presence and progression of CAD in diabetic patients is often asymptomatic, leading to more extensive disease at the time of diagnosis [2]. Since a delayed diagnosis of CAD considerably worsens the prognosis, early recognition of CAD could lead to more effectively targeted intervention and reduce morbidity and mortality in this population. Myocardial perfusion imaging (MPI) with SPECT is most commonly applied to identify patients with CAD and can accurately identify patients at increased cardiovascular risk [2-4]. However, based on recent data, a wide ranged routine MPI screening strategy of all asymptomatic patients with diabetes appears to be ineffective [5]. Accordingly, a selective "prescreening" strategy using an initial test for the identification of patients with a higher likelihood of abnormal MPI followed by referral of only these patients to MPI may be preferred. Non-invasive assessment of vascular stiffness could represent a promising tool for this purpose. In several studies, a relation between vascular stiffness and cardiovascular disease has been observed [6-8]. Assessment of the vascular stiffness, by means of pulse wave velocity (PWV) or pulse wave analysis (PWA) for augmentation index (AIx), may therefore have the potential to serve as a marker of abnormal MPI. Although PWV and AIx have been extensively studied in the general population [9-13], less data are available concerning their relation with CAD in asymptomatic patients with diabetes.

The aim of the current study was to prospectively assess the relation between the non-invasive measures of vascular stiffness (PWV and AIx) with the presence and extent of myocardial perfusion defects as assessed by SPECT MPI, in asymptomatic patients with diabetes.

METHODS

Study population

Prospectively, 160 consecutive asymptomatic patients with diabetes were recruited from a routine outpatient clinic. Patients were referred to the cardiology outpatient clinic for risk assessment and cardiovascular screening. Anginal symptoms were ruled out using a self-completed questionnaire for encountered chest pain [14]. The American Diabetes association (ADA) criteria were used to identify diabetes and for further stratification in type 1 or 2 diabetes [15]. Patients were considered as having type 1 diabetes if laboratory analysis demonstrated auto-antibodies to islet cells, insulin and glutamic acid decarboxylase or low levels of plasma c-peptide. Otherwise, patients were

considered to have type 2 diabetes. Medical history and demographics were obtained. All patients underwent physical examination, and blood and urine laboratory testing. MPI was performed as part of clinical work-up to determine presence and extent of myocardial perfusion defects. Additional measurements of PWV and AIx were used to assess vascular stiffness.

Cardiovascular risk factors

Cardiovascular risk factors were defined according to the following criteria: positive family history for CAD (presence of CAD in first degree family members male <55 years and / or female <65 years), smoking (current smoking or smoking in the last 2 years), hypertension (blood pressure >140/90 mmHg or treatment with antihypertensive medication), body mass index (kg/m^2), hypercholesterolemia (total cholesterol level >5.0 mmol/L or use of cholesterol lowering medication), and micro-albuminuria (urine albumin/creatinine ratio ≥3.5 mg/mmol) [2]. Plasma hemoglobin A1c was determined as a measure of glycemic control.

SPECT myocardial perfusion imaging

SPECT data acquisition

ECG-gated adenosine technetium-99m sestamibi (Tc99m MIBI) SPECT MPI was performed using a 2-day protocol, comprising of stress imaging on the first day and a rest scan on the second day [16]. Anti-hypertensive treatment with beta-adrenergic blocking agents or calcium antagonists was stopped and patients were instructed to abstain from caffeine containing products 24 hours prior to the stress test. Vasodilator stress was induced by intravenous infusion of adenosine 140 µg/kg/min for 6 min, with simultaneous handgrip exercise. Tc99m MIBI (500MBq) was injected intravenously after the third minute. Blood pressure and a 12-lead ECG were recorded throughout the adenosine infusion.

Images were acquired 2 hours after radiopharmaceutical injection using a triple head SPECT gamma camera (GCA 9300/HG; Toshiba Corporation, Tokyo, Japan) with low-energy, high-resolution collimators. Image acquisition was performed, using a circular 360˚ orbit, 60 projections, and 40 seconds per projection, in compliance with the American Society of Nuclear Cardiology (ASNC) imaging guidelines. Images were processed to obtain the short-axis, vertical long-axis, and horizontal long-axis sections, as well as polar map formats, normalized to maximal myocardial activity [16]. Patient motion was reviewed by examining the raw cine images. No attenuation or scatter correction was used.

SPECT data analysis

For semi-quantitative visual interpretation the myocardium was divided into 17 segments according to ASNC guidelines [16]. Tracer uptake in each segment was evaluated in consensus by two expert observers, blinded to patient's clinical characteristics and test results, using a 5-point scoring system ranging from 0 (normal uptake) to 4 (absent uptake). The summed stress score (SSS) was determined by the total sum of the 17 segmental scores of the stress images. MPI was considered normal if SSS <3. In case of abnormal MPI, a SSS 3-7 was considered to represent moderate MPI defects, and a SSS ≥8 to represent severe MPI defects [17]. Finally, regional wall motion on gated SPECT images was evaluated to allow differentiation between true MPI abnormalities and diaphragmatic or breast attenuation artifacts.

Assessment of Vascular Stiffness

Measurements were derived and analyzed non-invasively by applanation tonometry using a Sphygmocor system (SphygmoCor, Atcor Medical, Sydney, Australia). All measurements were performed in the morning in a quiet, temperature controlled clinical research laboratory by a specially trained technologist, blinded to patient's clinical characteristics and test results. Patients were instructed to abstain from their morning medication and remain fasting until after the test. Assessment of PWV and PWA commenced following a 10 minute rest in supine position, after a state of constant heart rate and blood pressure was reached.

Pulse wave velocity

The pulse waves were recorded at the common carotid artery and the femoral artery by sequential tonometry with simultaneous electrocardiographic gating. Pulse transit time was determined as the average of 10 consecutive beats. The distance between the two sites was measured. Aortic PWV (m/s) was defined as the distance between the 2 recording sites traveled by the pulse wave, divided by transit time. Using system software, aortic PWV was determined semi-automatically. The validation and reproducibility of this semi-automatic method have been previously published [18]. Measurements were performed three times in each patient and averaged to obtain the mean aortic PWV.

Pulse wave analysis

The peripheral pressure waveforms were recorded from the radial artery at the wrist, with a hand-held high fidelity tonometer (Millar Instruments, Houston, TX, USA) and calibrated by peripheral blood pressures of the brachial artery [7,19]. The corresponding central aortic pressure waveform was generated by a validated generalized transfer function. The central aortic pressure waveform was analyzed to identify the first shoulder

of the pressure wave representing the incident wave attributable to left ventricular ejection. The merging point of the incident and the reflected wave (the inflection point) was then identified on the generated aortic pressure waveform. The absolute augmented pressure was the maximum systolic pressure minus pressure at the inflection point. Subsequently, the AIx was defined as the absolute augmented pressure divided by the pulse pressure and expressed as a percentage [7,8].
Finally, the AIx was normalized for the heart rate of 75 bpm (AIx@75). For each patient, 3 consecutive waveform recordings were averaged to obtain the mean AIx@75, which was used for statistical analysis.

Statistical analysis
Continuous variables were expressed as means ± standard deviation and categorical variables as numbers (percentages). Firstly, associations of PWV and AIx@75 with baseline clinical risk factors were assessed using the Pearson's correlation coefficient (r) or the Spearman's rank correlation coefficient (r_s) in case of dichotomous variables. Secondly, differences in the mean PWV and AIx@75 for each group of MPI results were evaluated with the independent T-test. Thereafter, with univariate logistic regression analysis potential predictors of severe MPI defects were identified. Subsequently, all potential predictors were analyzed in a multivariate logistic regression model to identify the independent predictors of severe MPI defects. Additionally, patients were categorized according to PWV quartiles and for each quartile the prevalence of severe MPI defects was obtained. Subsequently, global chi square analysis was used to determine the incremental predictive value of PWV over baseline characteristics. Thereafter, using receiver operating characteristic (ROC) curve analysis two cut-off values were chosen for PWV; one cut-off value for the detection of severe MPI defects with optimal sensitivity and specificity and another cut-off value for the exclusion of severe MPI defects with optimal sensitivity and negative predictive value. All statistical analyses were performed using SPSS software (version 16.0, Inc., Chicago, Illinois). P-values <0.05 were considered statistically significant.

RESULTS

Patient characteristics
The study population comprised of 160 asymptomatic diabetic patients. Baseline characteristics are provided in Table 1.

Table 1 Baseline characteristics of the study population, n =160

Clinical factors	Mean ± st dev. or number (%)
Age	51 ± 12
Men	87 (54%)
Type 2 diabetes	91 (57%)
Diabetes duration (years)	15 ± 13
Insulin use	125 (78%)
Family history of CAD[a]	75 (47%)
Smoking	42 (26%)
Body mass index (kg/m²)	28 ± 6
HbA1c (mmol/l)[b]	8.2 ± 1.7
Hypertension	92 (58%)
Use of antihypertensive medication	76 (48%)
ACE-inhibitor use[c]	43 (27%)
Beta-blocker use	19 (12%)
Systolic blood pressure (mmHg)	133 ± 16
Diastolic blood pressure (mmHg)	80 ± 9
Hypercholesterolemia	107 (67%)
Cholesterol lowering medication	73 (46%)
Total cholesterol (mmol/l)	4.8 ± 1.1
Micro-albuminuria	39 (24%)
Aspirin use	31 (19%)

[a]CAD = coronary artery disease

[b]HbA1c = plasma hemoglobin A1c

[c]ACE = angiotensin converting enzyme

SPECT myocardial perfusion imaging

The overall mean SSS was 3.1 ± 4.1 (range 0-21). Abnormal MPI (SSS ≥3) was observed in 60 patients (38%), including moderate MPI defects (SSS 3-7) in 38 patients (24%) and severe MPI defects (SSS ≥8) in 22 patients (14%).

Vascular Stiffness

Pulse wave velocity

The overall mean PWV was 8.9 ± 2.4 m/s. PWV was associated with age (r = 0.62, P <0.01), type 2 diabetes (r_s = 0.23, P <0.01), diabetes duration (r = 0.30, P <0.01), body mass index (r = 0.22, P <0.01), hypertension (r_s = 0.43, P <0.01), and micro-albuminuria (r = 0.29, P <0.01).

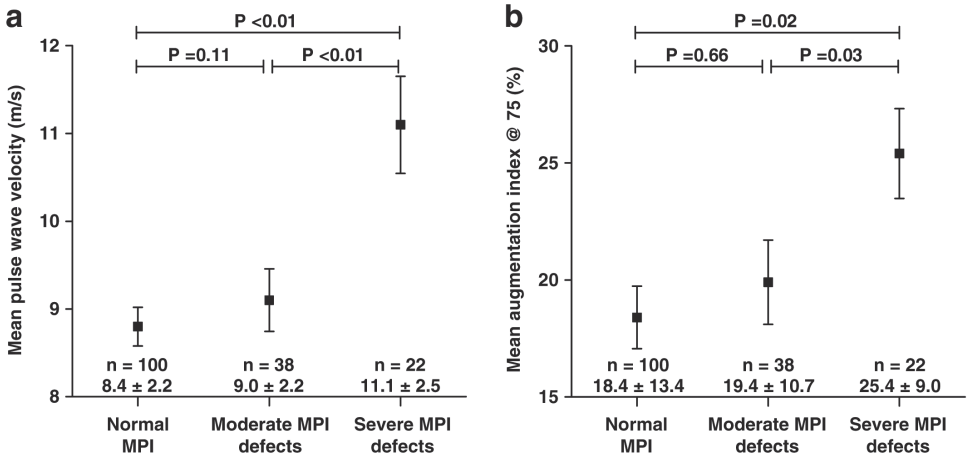

Figure 1 Relation between parameters of vascular stiffness and the extent of MPI defects as assessed by SPECT MPI. **a** Mean aortic PWV was increased in patients with abnormal MPI. The most substantial increase in PWV was observed in patients with severe MPI defects. **b** The relationship between mean AIx@75 and MPI shows a similar trend. Abbreviations: MPI = myocardial perfusion imaging and @ 75 = corrected for heart rate to 75 bpm.

As shown in Figure 1a, mean PWV increased only slightly from 8.4 ± 2.2 m/s in patients with normal MPI to 9.0 ± 2.2 m/s in patients with moderate MPI defects (P = 0.11). However, the mean PWV was significantly higher in patients with severe MPI defects (11.1 ± 2.5 m/s, P <0.01).

Association between pulse wave analysis with myocardial perfusion

The mean AIx was 21.1 ± 12.3% in the total population. Normalization for a heart rate of 75 beats per minute resulted in an overall mean AIx@75 of 19.6 ± 12.4%. A significant association was observed between AIx@75 and the following risk factors: age (r = 0.47, P <0.01), male gender (r_s = -0.43, P <0.01), type 2 diabetes (r_s = 0.30, P <0.01), hypercholesterolemia (r_s = 0.17, P <0.03), and micro-albuminuria (r = 0.26, P <0.01).

After stratification of mean AIx@75 according to SPECT MPI results, a trend similar to that of PWV was observed. Likewise, mean AIx@75 was slightly higher in patients with moderate MPI defects than in those with normal MPI (19.4 ± 10.7% and 18.4 ± 13.4% respectively, P = 0.66), and was significantly higher in patients with severe MPI (25.4 ± 9.0%, P = 0.03; Figure 1b).

Table 2 Predictors of severe MPI defects (SSS ≥8) on SPECT

Clinical characteristic	Exp β (95% CI)	P-value	Exp β (95% CI)	P-value
Age	1.09 (1.04-1.14)	<0.01	1.06 (0.98-1.14)	0.16
Male gender	3.30 (1.15-9.45)	0.03	6.35 (1.47-27.41)	0.01
Type 2 diabetes	1.47 (0.55-3.90)	0.44		
Diabetes duration (years)	1.02 (0.99-1.06)	0.15		
Family history of CAD[a]	1.16 (0.47-2.85)	0.75		
Smoking	3.80 (1.48-9.77)	0.01	5.74 (1.35-24.46)	0.02
Body mass index (kg/m^2)	0.99 (0.91-1.07)	0.77		
HbA1c (mmol/l)[b]	1.28 (1.00-1.65)	0.05	1.52 (1.03-2.25)	0.03
Hypertension	1.98 (0.73-5.41)	0.18		
Hypercholesterolemia	1.65 (0.57-4.79)	0.36		
Micro-albuminuria	3.86 (1.52-9.81)	0.01	1.05 (0.26-4.27)	0.95
PWV[c]	1.49 (1.22-1.81)	<0.01	1.49 (1.11-2.00)	0.01
AIx@75[d]	1.06 (1.01-1.11)	0.02	1.05 (0.97-1.14)	0.20

MPI = myocardial perfusion imaging

[a]CAD = coronary artery disease

[b]HbA1c = plasma hemoglobin A1c

[c]PWV = Pulse wave velocity (in m/s)

[d]AIx@75 = Augmentation index (%) normalized for the heart rate of 75 bpm.

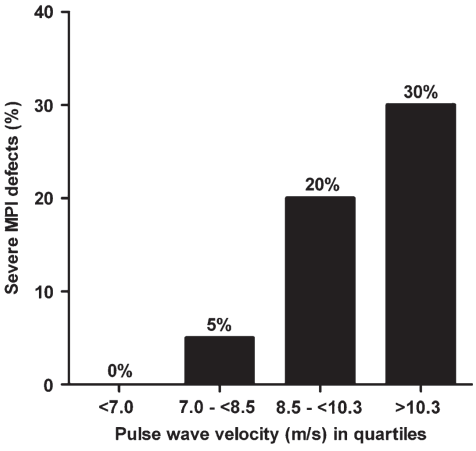

Figure 2 Prevalence of patients with severe MPI defects per PWV quartile. The prevalence of severe MPI defects increased with increasing PWV. Of note, the prevalence of severe MPI defects chiefly increased in the third and fourth PWV quartile. Abbreviations: MPI = myocardial perfusion imaging.

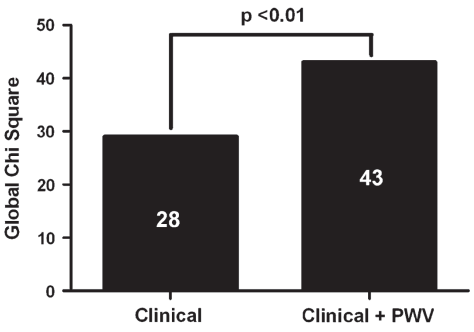

Figure 3 Incremental predictive value of PWV for the detection severe MPI defects as shown by an increase in global chi square. Addition of PWV to a model with baseline clinical risk factors age, gender, and smoking provided a significant incremental predictive value.

Predictors of severe myocardial perfusion defects

As illustrated in Table 2, age, gender, smoking, HbA1c, micro-albuminuria, and both PWV and AIx@75 were identified as potential predictors of severe MPI defects in a univariate logistic regression model. Of note, after adjustment for age, gender, smoking, HbA1c, and micro-albuminuria, the PWV remained a significant predictor of severe MPI defects (P = 0.01), whereas the AIx@75 was no longer significant.

As demonstrated in Figure 2, the prevalence of severe MPI defects gradually increased per PWV quartile. Importantly, in none of the patients in the lowest PWV quartile severe MPI defects were present. Also, only a relatively small proportion of patients (5%) in the second PWV quartile had severe MPI defects. In contrast, the prevalence of severe MPI defects increased to 20% in the third PWV quartile, while reaching 30% in the fourth quartile. Moreover, the addition of PWV to a model with baseline clinical risk factors age, gender, and smoking for the prediction of severe MPI defects showed significant incremental value of PWV (Figure 3).

ROC curve analysis for the detection of severe MPI defects showed highest sensitivity and specificity (77% and 75% respectively) with a PWV cut-off value of 9.8 m/s. An optimal sensitivity of 91% with an associated negative predictive value of 98% for the exclusion of severe MPI defects was found using a cut-off value of 9.2 m/s for PWV (Figure 4).

DISCUSSION

In the present study of asymptomatic patients with diabetes, vascular stiffness as assessed by PWV and AIx was increased in the presence of severe MPI defects. PWV was

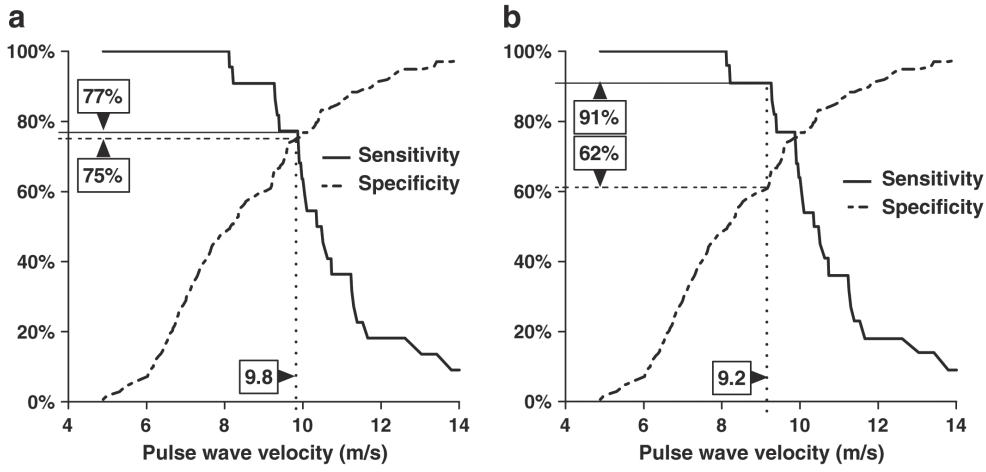

Figure 4 Detection of severe MPI defects on SPECT by PWV. **a** ROC curve analysis for the detection of severe MPI defects yielded an optimal sensitivity and specificity of 77% and 75% with a PWV cut-off value of 9.8 m/s. **b** In contrast, optimization for the exclusion of severe MPI defects resulted in a cut-off value of 9.2 m/s with a sensitivity of 91% and corresponding negative predictive value of 98%. ROC = receiver operating characteristics.

independently associated with severe MPI defects, whereas AIx lost significance after correction for other cardiovascular risk factors and PWV. Addition of PWV to a model of baseline clinical risk factors showed significant incremental value for the prediction of severe MPI defects. Furthermore, ROC curve analysis revealed a moderate to good sensitivity of 77% and a specificity of 75% for the detection of severe MPI defects, with a PWV cut-off value of 9.8 m/s. Conversely, changing the cut-off value to 9.2 m/s resulted in a high sensitivity of 91% and negative predictive value of 98% for the exclusion of severe MPI defects. Accordingly, the current results indicate that non-invasive evaluation of vascular stiffness may be a practical tool to prescreen asymptomatic patients with diabetes for the differentiation in a higher and lower likelihood of abnormal MPI.

Vascular stiffness and relationship to CAD

In the general population the relation between vascular stiffness and the presence of CAD has been confirmed in a considerable number of studies. Vascular stiffness measured as PWV or AIx is not only directly associated with the presence and severity of CAD on invasive coronary imaging [10], but also has incremental prognostic value for predicting cardiovascular events [9,11-13]. A recent meta-analysis (15877 subjects, 17 studies, average

follow-up of 7.7 years) showed that the risk of cardiovascular events increased a two-fold in patients with increased PWV [22]. Moreover, the predictive ability of PWV was shown to be even higher in patients with elevated baseline cardiovascular risk, supporting a role for PWV in high-risk populations such as patients with diabetes.

A few studies have specifically evaluated vascular stiffness in patients with diabetes. Cruickshank et al. evaluated the prognostic value of PWV for all-cause and cardiovascular mortality in 397 patients with diabetes with or without CAD. During a mean follow-up of 10.7 years, aortic PWV was an independent predictor for all-cause and cardiovascular mortality [23]. Additionally, Hatsuda et al. found in 595 patients with diabetes that PWV was significantly increased in 70 patients with established CAD [24]. Finally, Fukui et al. investigated 208 consecutive patients with type 2 diabetes and reported that AIx was significantly higher in 47 patients with previously confirmed CAD [21]. These observations indicate that markers of vascular stiffness may indeed be associated with CAD in patients with diabetes.

However, to our knowledge this is the first study in which PWV and AIx were applied in asymptomatic patients with diabetes to prospectively identify the presence of CAD defined by the presence of (severe) MPI defects. Although both PWV and AIx were increased in patients with severe MPI defects, only PWV was shown to be an independent predictor of severe MPI defects. These observations are in agreement with the previous literature as also in the general population more discrepant results have been reported using AIx as compared to PWV [25,26].

Possibly, the more variable results with AIx may be explained by underlying methodological differences. Carotid-femoral PWV is a direct measure of vascular stiffness as determined by the intrinsic stress/strain relationship of the arterial wall and mean arterial pressure. Therefore PWV is considered as the 'gold-standard' [6-8]. In contrast, AIx is an indirect measurement, derived from peripherally recorded pressure waveforms. Using a generalized transfer function the corresponding central arterial waveform is generated from which AIx is determined. Therefore, AIx is influenced by multiple factors such as PWV, heart rate, diastolic blood pressure, peripheral circulation, and endothelial function [7,8]. Furthermore, its discriminatory value may be less in the elderly [25,26], whereas also the use of the generalized transfer function may be inappropriate in certain populations [25-28]. In fact, Hope et al. recently evaluated the validity of this method found in patients with diabetes and revealed that estimation of central pressures was prone to substantially greater error in this population [27]. Similar differences in accuracy have also been reported in relation to gender, indicating that AIx might be a less representative marker of vascular stiffness as compared to PWV [25,26]. Conceivably, the weaker association between AIx and CAD as compared to PWV

may therefore be explained by the fact that our study was performed in patients with diabetes while also including a high percentage of female patients.

Clinical implications and perspectives

At present, screening of asymptomatic patients with diabetes for CAD remains controversial. Thus far, the majority of available data are based on CAD detection using SPECT MPI [3,4]. In the present study, the prevalence of abnormal MPI was 38%. In contrast, the recent DIAD trial demonstrated a much lower rate of abnormal MPI with only few patients having severe MPI defects [5,29]. To a large extent, this discrepancy may be explained by differences in baseline characteristics of the enrolled patients. Importantly, cardiac event rates were low in the DIAD study and not significantly reduced by a MPI based screening strategy. Nevertheless, in the small group of patients with abnormal MPI, a stepwise increase in event rates was observed with increasing MPI abnormality. Of note, hard event rates were 2% in patients with normal scans but increased to 12% in patients with at least moderately abnormal MPI scans. In contrast to the general asymptomatic diabetic population, these high risk patients may benefit from screening, as also suggested by the bypass angioplasty revascularization investigation BARI 2 diabetes trial [30,31]. In this trial no survival benefit was shown in patients undergoing early coronary revascularization as compared to intensive medical treatment. However, among high risk patients selected for coronary artery bypass grafting, prompt revascularization reduced major cardiovascular events as compared with medical therapy. Accordingly, these observations indicate that while routine screening for abnormal MPI may not be effective in asymptomatic patients with diabetes, selective screening strategies are warranted to identify the small but high risk subgroup within this population. In this regard, our current study may provide valuable data for the design of such strategies. Assessment of vascular stiffness by means of PWV was shown to accurately identify patients with a high-risk for severe MPI defects. Accordingly, further screening in patients with elevated PWV may be recommended. On the other hand, when using a slightly lower cut-off value, PWV was also shown to have a high negative predictive value, indicating that PWV can accurately rule out severe MPI defects. Therefore, further evaluation in patients with a negative PWV study may be safely omitted. Due to its low costs and non-invasive nature, PWV may represent a practical first-line tool to differentiate patients with a higher and lower likelihood of having abnormal MPI in this regard.

A number of limitations must be acknowledged in the current study. Some of the observed MPI defects may be attributed to artifact attenuation. However, regional wall motion on gated SPECT images was analyzed for optimal differentiation between true

MPI defects and attenuation artifacts. Evidently, larger prospective studies are needed to demonstrate the effectiveness of this strategy in terms of costs and outcome.

CONCLUSION

Vascular stiffness as non-invasively assessed by PWV is related with severely abnormal myocardial perfusion in asymptomatic patients with diabetes. Accordingly, PWV could be a practical tool to identify patients at higher risk for CAD and who could benefit from further screening.

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27(5):1047-53.
2. Consensus development conference on the diagnosis of coronary heart disease in people with diabetes: 10-11 February 1998, Miami, Florida. American Diabetes Association. Diabetes Care 1998;21(9):1551-9.
3. Kang X, Berman DS, Lewin HC, Cohen I, Friedman JD, Germano G, et al. Incremental prognostic value of myocardial perfusion single photon emission computed tomography in patients with diabetes mellitus. Am Heart J 1999;138(6 Pt 1):1025-32.
4. Miller TD, Rajagopalan N, Hodge DO, Frye RL, Gibbons RJ. Yield of stress single-photon emission computed tomography in asymptomatic patients with diabetes. Am Heart J 2004;147(5):890-6.
5. Young LH, Wackers FJ, Chyun DA, Davey JA, Barrett EJ, Taillefer R, et al. Cardiac outcomes after screening for asymptomatic coronary artery disease in patients with type 2 diabetes: the DIAD study: a randomized controlled trial. JAMA 2009;301(15):1547-55.
6. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. Circulation 2003;107(1):139-46.
7. Laurent S, Cockcroft J, Van Bortel L., Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 2006;27(21):2588-605.
8. Stehouwer CD, Henry RM, Ferreira I. Arterial stiffness in diabetes and the metabolic syndrome: a pathway to cardiovascular disease. Diabetologia 2008;51(4):527-39.
9. Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation 2006;113(5):657-63.
10. Weber T, Auer J, O'rourke MF, Kvas E, Lassnig E, Berent R, et al. Arterial stiffness, wave reflections, and the risk of coronary artery disease. Circulation 2004;109(2):184-9.
11. Weber T, Auer J, O'rourke MF, Kvas E, Lassnig E, Lamm G, et al. Increased arterial wave reflections predict severe cardiovascular events in patients undergoing percutaneous coronary interventions. Eur Heart J 2005;26(24):2657-63.
12. Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, et al. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. Circulation 2006;113(9):1213-25.
13. Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H, et al. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation 2006;113(5):664-70.

14. Rose G, McCartney P, Reid DD. Self-administration of a questionnaire on chest pain and intermittent claudication. Br J Prev Soc Med 1977;31(1):42-8.
15. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003;26 Suppl 1:S5-20.
16. Hansen CL, Goldstein RA, Akinboboye OO, Berman DS, Botvinick EH, Churchwell KB, et al. Myocardial perfusion and function: single photon emission computed tomography. J Nucl Cardiol 2007;14(6):e39-e60.
17. Petretta M, Acampa W, Evangelista L, Daniele S, Ferro A, Cuocolo A. Impact of inducible ischemia by stress SPECT in cardiac risk assessment in diabetic patients: rationale and design of a prospective, multicenter trial. J Nucl Cardiol 2008;15(1):100-4.
18. Wilkinson IB, Fuchs SA, Jansen IM, Spratt JC, Murray GD, Cockcroft JR, et al. Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. J Hypertens 1998;16(12 Pt 2):2079-84.
19. Oliver JJ, Webb DJ. Noninvasive assessment of arterial stiffness and risk of atherosclerotic events. Arterioscler Thromb Vasc Biol 2003;23(4):554-66.
20. Cecelja M, Chowienczyk P. Dissociation of aortic pulse wave velocity with risk factors for cardiovascular disease other than hypertension: a systematic review. Hypertension 2009;54(6):1328-36.
21. Fukui M, Kitagawa Y, Nakamura N, Mogami S, Ohnishi M, Hirata C, et al. Augmentation of central arterial pressure as a marker of atherosclerosis in patients with type 2 diabetes. Diabetes Res Clin Pract 2003;59(2):153-61.
22. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol 2010;55(13):1318-27.
23. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation 2002;106(16):2085-90.
24. Hatsuda S, Shoji T, Shinohara K, Kimoto E, Mori K, Fukumoto S, et al. Regional arterial stiffness associated with ischemic heart disease in type 2 diabetes mellitus. J Atheroscler Thromb 2006;13(2):114-21.
25. Fantin F, Mattocks A, Bulpitt CJ, Banya W, Rajkumar C. Is augmentation index a good measure of vascular stiffness in the elderly? Age Ageing 2007;36(1):43-8.
26. Hope SA, Antonis P, Adam D, Cameron JD, Meredith IT. Arterial pulse wave velocity but not augmentation index is associated with coronary artery disease extent and severity: implications for arterial transfer function applicability. J Hypertens 2007;25(10):2105-9.

27. Hope SA, Tay DB, Meredith IT, Cameron JD. Use of arterial transfer functions for the derivation of central aortic waveform characteristics in subjects with type 2 diabetes and cardiovascular disease. Diabetes Care 2004;27(3):746-51.
28. Lacy PS, O'Brien DG, Stanley AG, Dewar MM, Swales PP, Williams B. Increased pulse wave velocity is not associated with elevated augmentation index in patients with diabetes. J Hypertens 2004;22(10):1937-44.
29. Wackers FJ, Young LH, Inzucchi SE, Chyun DA, Davey JA, Barrett EJ, et al. Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. Diabetes Care 2004;27(8):1954-61.
30. Chaitman BR, Hardison RM, Adler D, Gebhart S, Grogan M, Ocampo S, et al. The Bypass Angioplasty Revascularization Investigation 2 Diabetes randomized trial of different treatment strategies in type 2 diabetes mellitus with stable ischemic heart disease: impact of treatment strategy on cardiac mortality and myocardial infarction. Circulation 2009;120(25):2529-40.
31. Frye RL, August P, Brooks MM, Hardison RM, Kelsey SF, MacGregor JM, et al. A randomized trial of therapies for type 2 diabetes and coronary artery disease. N Engl J Med 2009;360(24):2503-15.

CHAPTER 10

Incremental Value of Coronary Artery Calcium Score over Micro-Albuminuria to predict Myocardial Ischemia in Asymptomatic Patients with Type 1 Diabetes

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Submitted

ABSTRACT

Aim

Patients with type 1 diabetes are often only considered at high risk for myocardial ischemia in the presence of micro-albuminuria. However, limited data are available on the prevalence and predictors of myocardial ischemia in this population and possibly more refinement is needed. We evaluated the presence and extent of myocardial ischemia, as well as the predictive value of micro-albuminuria and coronary artery calcium (CAC) scores, in asymptomatic patients with type 1 diabetes.

Methods

Prospectively, clinical characteristics and CAC scores were obtained in 83 asymptomatic patients with type 1 diabetes. Myocardial ischemia was evaluated by determining the presence and extent of myocardial perfusion defects on SPECT myocardial perfusion imaging. The relation between clinical characteristics, including micro-albuminuric status, and CAC scores, with the extent of myocardial perfusion defects was analyzed.

Results

Myocardial ischemia was observed in 34 patients (41%) with 12 patients (14%) having severe perfusion defects. Significant predictors of extent of perfusion defects were age, micro-albuminuria and a CAC score >100. Nevertheless, severe perfusion defects were observed in 7 patients (9%) without micro-albuminuria, whereas a CAC score <100 excluded severe perfusion defects. Additionally, CAC scoring was shown to have incremental value for predicting severe perfusion defects over age and the presence of micro-albuminuria ($P = 0.01$).

Conclusions

In asymptomatic patients with type 1 diabetes, myocardial ischemia was observed in a substantial number of patients. Predictors of the extent of ischemia were micro-albuminuria and a CAC score >100. However, CAC score assessment was superior to micro-albuminuria in exclusion of severe myocardial perfusion defects.

INTRODUCTION

Cardiovascular disease is the major cause of mortality not only in type 2 – but also in type 1 diabetes (1,2). Compared with the general population, coronary artery disease (CAD) as reflected by the presence of myocardial ischemia, occurs in patients with type 1 diabetes earlier in life (3). To suppress the premature progression of atherosclerosis, AHA/ADA guidelines for primary prevention advise stringent pharmacological therapy including statin therapy and anti-hypertensive medication in all diabetic patients regardless of the type of diabetes (4). However, based on previous observations indicating that excess cardiovascular mortality in type 1 diabetes predominantly occurs in patients with nephropathy, European guidelines consider patients with type 1 diabetes only as being at high risk for myocardial ischemia in presence of micro-albuminuria (5). Nonetheless, uncertainty exists regarding the degree to which renal disease influences the presence and extent of myocardial ischemia in type 1 diabetes. Furthermore, recent studies suggest persistent risk of myocardial ischemia in type 1 diabetes despite adjustment for micro-albuminuria (6). As a result, the effectiveness of micro-albuminuric status as a selection strategy to identify type 1 diabetic patients at high risk for myocardial ischemia is questioned and additional markers may be needed. To improve identification of patients at risk for myocardial ischemia, the use of direct markers of coronary atherosclerosis, such as coronary artery calcium (CAC) scoring has been evaluated in patients with type 2 diabetes (7-9). However, thus far only limited data are available on both the prevalence of myocardial ischemia and potential clinical markers to predict myocardial ischemia in type 1 diabetes (10,11). In order to optimize guidelines for management of asymptomatic type 1 diabetic patients more data are required.

The purpose of the present study was therefore to explore the prevalence and extent of abnormal myocardial perfusion (as a marker of myocardial ischemia) as assessed by SPECT in asymptomatic patients with type 1 diabetes. In addition, the value of clinical factors including micro-albuminuric status as well as CAC scoring for predicting the presence and extent of abnormal myocardial perfusion was evaluated.

METHODS

Patients and design

Eighty-three consecutive asymptomatic patients with type 1 diabetes were prospectively recruited from a routine diabetes clinic, and referred to the cardiology outpatient clinic for cardiovascular screening. Asymptomatic status was confirmed using the self-administered Rose questionnaire for angina (12). Baseline clinical demographics were acquired through a structured interview, physical examination and laboratory analysis. For this purpose, cardiovascular risk factors were assessed in compliance

with the following criteria: 1. positive family history of CAD (defined as presence of CAD in first degree family members at <55 years in men and <65 years in women), 2. smoking (defined as current smoking or smoking in the last 2 years), 3. body mass index (BMI) (kg/m^2), 4. level of glycemic control represented by glycated-haemoglobin A1c (HbA1c) (mmol/L), 5. hypertension (defined as a blood pressure >140/90 mmHg or treatment with antihypertensive medication), 6. hypercholesterolemia (defined as a total cholesterol level >5.0 mmol/L or use of cholesterol lowering medication) and 7. micro-albuminuria (defined as urine albumin/creatinine ratio ≥3.5 mg/mmol).
All patients underwent assessment of CAC scores by means of Multi-Slice Computed Tomography (MSCT) and myocardial perfusion imaging by SPECT as part of their clinical evaluation.

Coronary artery calcium score – data acquisition
CAC scoring was performed with a 64-slice MSCT scanner (Aquilion64, Toshiba Medical Systems, Tokyo, Japan). If necessary and tolerated, oral beta-blockers (metoprolol 50 mg or 100 mg) were provided 1 hour preceding the scan to achieve a heart rate <65 beats per minute. Thereafter, a non-enhanced prospective electrocardiographically gated scan, triggered at 75% of the R-R interval with 4 x 3.0 mm collimation was obtained to measure CAC score.

Coronary artery calcium score - data analysis
Data were evaluated with a remote workstation using dedicated software (Vitrea2, Vital Images, Minnetonka, USA). In each patient, coronary calcium was identified as a dense area in the coronary artery exceeding the threshold of 130 Hounsfield units. The total Agatston score was determined for each patient. Patients with a CAC score >100 were classified as having increased CAC (13).

ECG-gated SPECT - data acquisition
During a two-day stress and rest protocol, myocardial perfusion imaging to detect myocardial ischemia was performed using ECG-gated SPECT with ^{99m}Tc sestamibi (^{99m}TcMIBI)(8). Patients were instructed to abstain from caffeine containing products for 24 hours, preceding the stress test. Stress was induced using intravenous infusion of adenosine at a rate of 140 µg/kg body weight per minute for 6 minutes, accompanied by simultaneous handgrip exercise. After completion of the third minute ^{99m}TcMIBI (500 MBq) was injected intravenously. Blood pressure and a 12-lead ECG were recorded throughout the adenosine infusion. Imaging commenced 120 minutes after radiopharmaceutical injection using a triple-head SPECT gamma camera (GCA 9300/HG; Toshiba Corporation, Tokyo, Japan) equipped with low-energy high-resolution

collimators. Image acquisition was performed in accordance with American Society of Nuclear Cardiology (ASNC) guidelines, using a circular 360° orbit, 60 projections, and 40 seconds per projection. Attenuation correction was not applied. Images were processed in the usual manner and short-axis, horizontal long-axis and vertical long-axis views were reconstructed. Patient motion was evaluated by examining the raw cine images.

ECG-gated SPECT - data analysis

Short-axis slices were displayed in polar map format, adjusted for peak myocardial activity of 100%. Additional reconstruction yielded standard long- and short-axis projections perpendicular to the heart axis. All views were used for semi-quantitative interpretation. As proposed by the ASNC guidelines, the myocardium was divided into 17 segments (14). Tracer uptake in each segment was evaluated by two observers in consensus, by use of a 5-point scoring system (0: normal, 1: mild, 2: moderate, 3: severe reduction of tracer uptake and 4: apparent absence of tracer uptake). The total segmental score during stress and rest was used to determine the summed stress score (SSS) and summed rest score (SRS) for each patient. Presence of a myocardial perfusion defect was defined as SSS≥3, and severe myocardial perfusion defects were defined as SSS≥8 (corresponding with approximately ≥10% of myocardium) (15,16). Furthermore, regional wall motion on gated SPECT images was analyzed to differentiate between true perfusion abnormalities and attenuation artifacts (14).

In addition, data were evaluated for other abnormalities indicative of myocardial ischemia (14). Ischemic ECG changes were defined as a down-sloping or depression of the ST-segments greater than 1 mm following the J-point in two or more leads during stress. Left ventricular ejection fraction (LVEF) at rest and during stress was derived from the end-diastolic volume (EDV) and end-systolic volume (ESV). Patients with a LVEF <45% in rest were stratified as having left ventricular dysfunction. Furthermore, SPECT images revealing increased radiotracer lung-uptake or transient ischemic dilation (TID) (defined as a stress- and rest short axis volume ratio larger than 1.21) were also classified as abnormal.

Finally, the overall SPECT myocardial perfusion imaging study result was considered abnormal in presence of any of the following abnormalities: 1) abnormal myocardial perfusion (SSS ≥3), 2) ischemic ECG changes, 3) left ventricular dysfunction, 4) increased lung-uptake, 5) TID (14).

Statistical Analysis

Continuous variables were expressed as means ± standard deviation. Categorical variables were expressed as numbers (percentages).

First, the prevalence of 1. an abnormal SPECT myocardial perfusion imaging study, 2. myocardial perfusion defects and 3. severe myocardial perfusion defects were determined.
Second, univariate linear regression analysis of baseline clinical characteristics was performed to identify potential predictors of the extent of myocardial perfusion defects represented by SSS. Thereafter, clinical characteristics with a *P* value <0.05 were included in a linear multiple regression model to identify independent predictors of SSS. In a next step, the relation between the presence of micro-albuminuria and a CAC score >100 with abnormal myocardial perfusion was further studied. To this end, the distribution of patients with 1. an abnormal SPECT myocardial perfusion imaging study, 2. myocardial perfusion defects and 3. severe myocardial perfusion defects, was first compared according to presence of micro-albuminuria. The analysis was then repeated according to the presence of a CAC score >100.
Finally, the incremental value of micro-albuminuria and presence of a CAC score >100 over other observed clinical predictors of severe myocardial perfusion defects (namely age) was assessed by calculating the global chi-square test.
Statistical analyses were performed using SPSS software (version 16.0.1, Inc., Chicago, Illinois). P values <0.05 were considered statistically significant.

RESULTS

Patient characteristics

In total, 83 consecutive asymptomatic patients with type 1 diabetes were referred to the cardiology outpatient clinic and included in the analysis. The baseline clinical demographics of the study population are provided in Table 1. Briefly, mean age was 48 ± 12 years and the majority of the patients were male (63%). The mean duration of diabetes was 23 ± 12 years. At the time of referral to the cardiology outpatient clinic, 37% of the patients received statin therapy, 19% were treated with aspirin and 27% of the patients were treated with ACE-inhibitors.

Coronary artery calcium scoring

Overall, the CAC score ranged from 0 to 2773 with a mean CAC score of 214 ± 510. In total, 57 patients (69%) had a CAC score in the range 0-100, whereas an increased CAC score (>100) was observing in 26 patients (31%).

SPECT Myocardial perfusion imaging

Mean SSS was 3.1 ± 4.2 in the total population. Overall, 34 patients (41%) had an abnormal SPECT myocardial perfusion study reflecting the presence of myocardial ischemia.

Table 1. Characteristics of the study population

Age (years)	48 ± 12
Male sex	52 (63%)
Diabetes duration (years)	23 ± 13
Family history of CAD	35 (42%)
Smoking	24 (29%)
BMI (kg/m^2)	25 ± 4
HbA1c (mmol/L)	7.9 ± 1.5
Hypercholesterolemia	51 (61%)
Hypertension	43 (52%)
Cardiovascular medication use at the time of referral	
Statin	31 (37%)
Aspirin	16 (19%)
ACE-inhibitor	22 (27%)

Data are averages ± standard deviation or numbers (%).
BMI = body mass index, CAD = coronary artery disease.

Herein, 27 patients (33%) had myocardial perfusion defects (SSS ≥3), including reversible defects in 11 patients (41%), partially reversible defects in 9 patients (33%) and fixed defects in 7 patients (26%). Importantly, in 12 patients (14%), severe myocardial perfusion defects (SSS ≥8) were observed.

Additionally, SPECT data analysis revealed other abnormalities indicative of myocardial ischemia in 7 patients (8%) with normal myocardial perfusion: 2 patients (2%) had ischemic ECG changes during adenosine stress, another 2 patients (2%) were diagnosed with left ventricular dysfunction, and 3 patients (4%) were shown to have TID.

Predictors of the extent of myocardial perfusion defects

Using univariate linear regression analysis, age, presence of micro-albuminuria and a CAC score >100 were identified as significant predictors of the extent of myocardial perfusion defects represented by SSS (Table 2). Analysis of these risk factors in a multivariate linear regression model revealed micro-albuminuria and CAC score >100 to be independent predictors of the extent of myocardial perfusion defects on SPECT (Table 2).

Relation of micro-albuminuria with myocardial perfusion

As illustrated in Figure 1, the prevalence of an abnormal SPECT myocardial perfusion imaging study was significantly higher in patients with micro-albuminuria (77%) as compared to those without micro-albuminuria (34%) ($P = 0.004$). Similarly, the prevalence

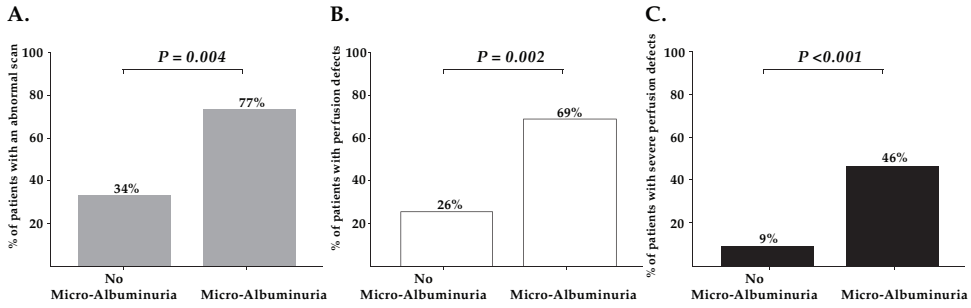

Figure 1. Bar graphs illustrating the relation between micro-albuminuria and SPECT myocardial perfusion imaging. Patients with micro-albuminuria were shown to have a higher prevalence of abnormal SPECT myocardial perfusion imaging studies (panel A), myocardial perfusion defects (panel B) and severe myocardial perfusion defects (panel C). However, the absence of micro-albuminuria did not completely exclude the presence of severe myocardial perfusion defects (panel C).

of myocardial perfusion defects was higher in presence of micro-albuminuria (69%) than in its absence (26%) (P = 0.002). Finally, also severe myocardial perfusion defects were more often observed in the presence of micro-albuminuria (P <0.001). However, severe perfusion defects were still noted in 9% of patients without micro-albuminuria.

Relation of coronary artery calcium scores with myocardial perfusion

Relation of CAC scores with SPECT data are provided in Figure 2. The presence of an increased CAC score (>100) as compared to a CAC score 0-100 was associated with a higher prevalence of abnormal SPECT myocardial perfusion imaging studies (63% vs 30%) (P = 0.005) and a higher prevalence of myocardial perfusion defects (59% vs 20%) (P <0.001). Importantly, severe myocardial perfusion defects were absent in patients with a CAC score 0-100. In contrast, almost half (44%) of patients with an increased CAC score (>100) showed severe myocardial perfusion defects (P <0.001).

Incremental value of micro-albuminuria and CAC score in the prediction of severe myocardial perfusion defects

The incremental value for prediction of severe myocardial perfusion defects was analyzed for micro-albuminuria and an increased CAC score (>100) over age (the only other clinical risk factor predictive of the extent of myocardial perfusion defects). For this purpose global chi-square scores were calculated and are presented in Figure 3. The presence of micro-albuminuria was shown to have incremental value over age (P = 0.01)

Table 2. Predictors of the extent of myocardial perfusion defects (SSS)

	Univariate Analysis		Multivariate Analysis	
	Exp ß (95% CI)	*P Value*	*Exp ß (95% CI)*	*P Value*
Age (years)	0.10 (0.02-0.18)	0.01	0.01 (-0.07-0.09)	0.77
Male sex	0.68 (-1.22-2.57)	0.48	-	-
Diabetes duration (years)	0.03 (-0.04-0.09)	0.48	-	-
BMI (kg/m^2)	0.11 (-0.12-0.35)	0.34	-	-
HbA1c (mmol/L)	0.08 (-0.55-0.70)	0.81	-	-
Smoking	1.56 (-0.46-3.58)	0.13	-	-
Family history of CAD	-1.22 (-3.06-0.63)	0.19	-	-
Hypercholesterolemia	0.98 (-0.90-2.86)	0.30	-	-
Hypertension	1.26 (-0.59-3.10)	0.18	-	-
Micro-albuminuria	5.04 (2.77-7.32)	<0.001	3.19 (0.84-5.54)	0.008
Statin use	-0.21 (-2.12-1.69)	0.83	-	-
Aspirin use	1.15 (-1.17-3.47)	0.33	-	-
ACE-inhibitor use	1.49 (-0.57-3.55)	0.15	-	-
CAC score >100	4.51 (2.79-6.23)	<0.001	3.30 (1.17-5.43)	0.003

BMI = body mass index, CAC = coronary artery calcium and CAD = coronary artery disease.

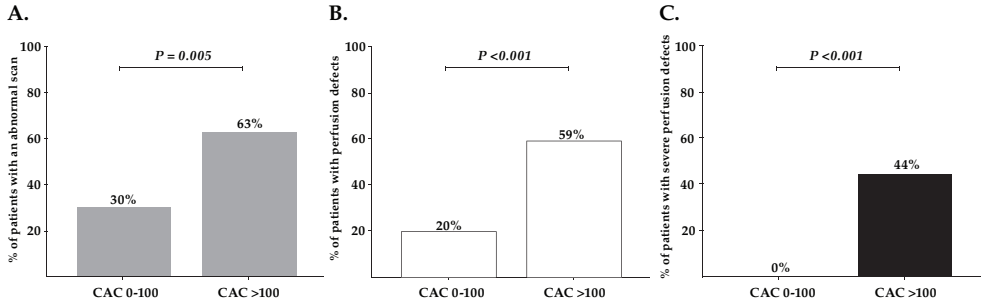

Figure 2. Bar graphs illustrating the relation between CAC score and SPECT myocardial perfusion imaging. As compared to patients with a low CAC score (0-100), patients with a CAC score >100, were shown to have a higher prevalence of abnormal SPECT myocardial perfusion imaging studies (panel A), myocardial perfusion defects (panel B) and severe myocardial perfusion defects (panel C). A CAC score 0-100 successfully excluded the presence of severe myocardial perfusion defects (panel C).

in the prediction of severe myocardial perfusion defects. Importantly the addition of CAC scores provided further information, incremental over the combination of age and micro-albuminuric status (P = 0.01).

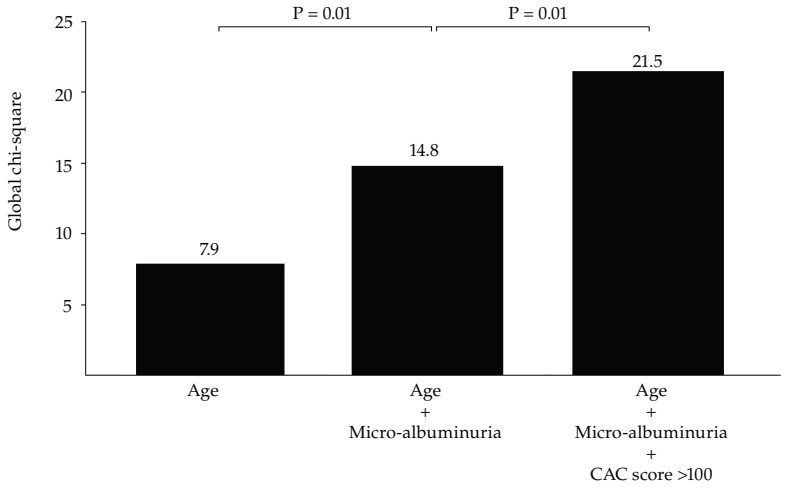

Figure 3. Bar graph illustrating the incremental value of micro-albuminuria and a CAC score >100 in predicting severe myocardial perfusion defects. The addition of micro-albuminuric status provided incremental information over age. Furthermore, the addition of CAC score >100 resulted in further incremental information over age and micro-albuminuric status.

DISCUSSION

In the current study of asymptomatic patients with type 1 diabetes, an abnormal SPECT myocardial perfusion imaging study reflecting myocardial ischemia was obtained in 41% of patients. Moreover, severe myocardial perfusion defects were detected in a considerable number of patients (14%). The presence of micro-albuminuria, which is currently proposed as a criterion for stringent pharmacological therapy in type 1 diabetes by the European prevention guidelines, was indeed associated with the extent of myocardial perfusion defects. In particular, 77% of patients with micro-albuminuria had an abnormal myocardial perfusion imaging study while severe myocardial perfusion defects were observed in 46% of patients with micro-albuminuria. However, the absence of micro-albuminuria could not exclude severe myocardial perfusion defects. Almost 10% of patients without micro-albuminuria still had severe myocardial perfusion defects, indicating the need for additional clinical markers to determine cardiovascular risk more precisely. In this regard, CAC scoring was demonstrated to have incremental value for the prediction of severe myocardial perfusion defects. Importantly, a low CAC score (0-100) was shown to successfully rule out severe myocardial perfusion defects on SPECT in all patients.

Myocardial perfusion imaging by SPECT

In the general population of patients with known or established CAD, SPECT myocardial perfusion imaging has been shown to accurately identify the presence of myocardial ischemia (as a marker of CAD) and predict future cardiac events (17). Similarly, the prognostic value of SPECT has been confirmed in studies of heterogeneous populations of type 1 and type 2 diabetic patients with known or established CAD (17). In particular, severe ischemia involving a large proportion of the left ventricular myocardium has been associated with occurrence of adverse cardiac events (18). The prevalence, extent and clinical predictors of myocardial ischemia have been evaluated in relatively large cohorts of asymptomatic patients with type 2 diabetes (7-9). However, limited data are available on the prevalence and extent of silent myocardial ischemia in type 1 diabetes (10,11). In a small population of patients with type 1 diabetes scheduled for islet transplantation, SPECT imaging revealed myocardial perfusion defects in only a minority of patients (9%), although a substantially higher prevalence of obstructive coronary stenoses (43%) was observed during conventional coronary angiography (11). Possibly, differences in study design and definitions of an abnormal myocardial perfusion study account for the discrepancies in observed results and limit direct comparison between the studies. In the current study, abnormal myocardial perfusion was defined and quantified according to guidelines proposed by the ASNC (14). Using this approach, we observed a high prevalence of abnormal SPECT myocardial perfusion imaging studies (41%). Notably, severe myocardial perfusion defects were identified in 14% of patients, underlining the notion that patients with type 1 diabetes may be at higher risk as compared to the general population (1,2).

Current guidelines for primary prevention and risk stratification in type 1 diabetes

The high risk of mortality from CAD in type 1 diabetes has been recognized in the Diabetes UK Cohort study (19). As compared to the general population, cardiovascular mortality was increased by a 6 fold in type 1 diabetic men and a 15 fold in women, emphasizing the need to identify and treat CAD in this cohort. To reduce the progression and clinical impact of CAD, the AHA/ADA guidelines for primary prevention therefore recommend pharmacological treatment of cardiovascular risk factors in all asymptomatic patients with type 1 diabetes (4). In contrast, European guidelines consider type 1 diabetic patients to be at higher risk only in presence of micro-albuminuria (5). This recommendation is based on previous studies which identified diabetic nephropathy as a main predictor of cardiovascular prognosis in patients with type 1 diabetes (20-23). Indeed, a strong relation has been shown between nephropathy in type 1 diabetic patients and the extent of coronary and aortic atherosclerosis as assessed by magnetic resonance imaging (24). Increasing evidence suggests that these two complications of type 1 diabetes, share risk

factors such as hyperglycaemia and consequent endothelial dysfunction, and therefore develop among similar path-lines (25-27). The results of the current study also support the importance of micro-albuminuria as a risk factor, as its presence was significantly related with the extent of myocardial ischemia. However, myocardial perfusion defects, as well as severe myocardial perfusion defects were observed even in absence of micro-albuminuria. Thus, use of micro-albuminuria as the sole criterion for further risk stratification of asymptomatic patients with type 1 diabetes may underestimate preclinical myocardial ischemia and result in insufficient treatment. Possibly, additional clinical markers may be needed to identify patients with type 1 diabetes that are at elevated risk for myocardial ischemia.

Risk stratification using coronary artery calcium scoring

CAC scoring is a well-established, non-invasive technique to identify coronary atherosclerotic plaque burden, both in the general population (28) and in patients with diabetes (29). Furthermore, Anand and colleagues have previously described the potential of CAC scoring in the prediction of silent myocardial ischemia in type 2 diabetic patients (30). Hence, evaluation of the CAC score has been suggested as a primary step in risk stratification of patients with type 2 diabetes (31). Interestingly, recent data suggest that CAC scores may be even more accurate in the identification of coronary atherosclerosis in patients with type 1 diabetes as compared to type 2 diabetes (32,33). In line with these observations, the current study of asymptomatic patients with type 1 diabetes showed that the presence of an increased CAC score (>100) was a significant predictor of the extent of myocardial perfusion defects. Of note, in patients with limited coronary calcium (CAC score 0-100), the prevalence of abnormal myocardial perfusion was low. Furthermore, the presence of a severe myocardial perfusion defect could be reliably ruled out based on the absence of increased CAC. In addition, CAC scores were shown to provide incremental value over age and micro-albuminuric status in the prediction of severe myocardial perfusion defects. While patients with micro-albuminuria are considered to be at high risk, CAC scores may be of particular value in patients without micro-albuminuria. In these patients, knowledge of the extent of CAC may allow more accurate separation of patients at either low or high risk of myocardial ischemia.

Importantly, prospective studies in preferably larger cohorts should address the prognostic implications of our observations.

CONCLUSION

In asymptomatic patients with type 1 diabetes, abnormal myocardial perfusion imaging studies, including the presence of severe myocardial perfusion defects, were observed in a substantial number of patients. The presence of micro-albuminuria and increased CAC scores (>100) were shown to be independent predictors of the extent of myocardial perfusion defects. However, CAC score assessment was superior to micro-albuminuric status in the exclusion of severe myocardial perfusion defects.

REFERENCES

1. Soedamah-Muthu SS, Fuller JH, Mulnier HE, Raleigh VS, Lawrenson RA, Colhoun HM (2006) All-cause mortality rates in patients with type 1 diabetes mellitus compared with a non-diabetic population from the UK general practice research database, 1992-1999. Diabetologia 49:660-666
2. Juutilainen A, Lehto S, Rönnemaa T, Pyörälä K, Laakso M (2008) Similarity of the impact of type 1 and type 2 diabetes on cardiovascular mortality in middle-aged subjects. Diabetes Care 31:714-719
3. Valsania P, Zarich SW, Kowalchuk GJ, Kosinski E, Warram JH, Krolewski AS (1991) Severity of coronary artery disease in young patients with insulin-dependent diabetes mellitus. Am Heart J 122:695-700
4. Buse JB, Ginsberg HN, Bakris GL et al; American Heart Association; American Diabetes Association (2007) Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. Diabetes Care 30:162-172
5. De Backer G, Ambrosioni E, Borch-Johnsen K et al; Third Joint Task Force of European and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (2003) European guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J 24:1601-1610
6. Nathan DM, Cleary PA, Backlund JY et al; Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group (2005) Intensive diabetes treatment and cardiovascular disease in patients with type 1. N Engl J Med 353:2643-2653
7. Rajagopalan N, Miller TD, Hodge DO, Frye RL, Gibbons RJ (2005) Identifying high-risk asymptomatic diabetic patients who are candidates for screening stress single-photon emission computed tomography imaging. J Am Coll Cardiol 45:43-49
8. Wackers FJ, Young LH, Inzucchi SE et al; Detection of Ischemia in Asymptomatic Diabetics Investigators (2004) Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. Diabetes Care 27:1954-1961
9. Zellweger MJ, Hachamovitch R, Kang X et al (2004) Prognostic relevance of symptoms versus objective evidence of coronary artery disease in diabetic patients. Eur Heart J 25:543-550
10. Libby P, Nathan DM, Abraham K et al (2005) Report of the National Heart, Lung, and Blood Institute-National Institute of Diabetes and Digestive and Kidney Diseases Working Group on Cardiovascular Complications of Type 1 Diabetes Mellitus. Circulation 111:3489-3493
11. Senior PA, Welsh RC, McDonald CG, Paty BW, Shapiro AM, Ryan EA (2005) Coronary artery disease is common in nonuremic, asymptomatic type 1 diabetic islet transplant candidates. Diabetes Care 28:866-872

12. Rose G, McCartney P, Reid DD (1977) Self-administration of a questionnaire on chest pain and intermittent claudication. Br J Prev Soc Med 31:42-48
13. Greenland P, Bonow RO, Brundage BH et al; American College of Cardiology Foundation Clinical Expert Consensus Task Force (ACCF/AHA Writing Committee to Update the 2000 Expert Consensus Document on Electron Beam Computed Tomography); Society of Atherosclerosis Imaging and Prevention; Society of Cardiovascular Computed Tomography (2007) ACCF/AHA 2007 clinical expert consensus document on coronary artery calcium scoring by computed tomography in global cardiovascular risk assessment and in evaluation of patients with chest pain: a report of the American College of Cardiology Foundation Clinical Expert Consensus Task Force (ACCF/AHA Writing Committee to Update the 2000 Expert Consensus Document on Electron Beam Computed Tomography). Circulation 115:402-426
14. Hansen CL, Goldstein RA, Akinboboye OO, Berman DS, Botvinick EH, Churchwell KB, Cooke CD, Corbett JR, Cullom SJ, Dahlberg ST, Druz RS, Ficaro EP, Galt JR, Garg RK, Germano G, Heller GV, Henzlova MJ, Hyun MC, Johnson LL, Mann A, McCallister BD Jr, Quaife RA, Ruddy TD, Sundaram SN, Taillefer R, Ward RP, Mahmarian JJ; American Society of Nuclear Cardiology (2007) Myocardial perfusion and function: single photon emission computed tomography. J Nucl Cardiol 14:e39-60
15. Hachamovitch R, Hayes SW, Friedman JD, Cohen I, Berman DS (2003) Comparison of the short-term survival benefit associated with revascularization compared with medical therapy in patients with no prior coronary artery disease undergoing stress myocardial perfusion single photon emission computed tomography. Circulation 107:2900-2907
16. Iskandrian AE (1999) Risk assessment of stable patients (Panel II): proceedings of the 4th Invitational Wintergreen Conference Wintergreen panel summaries. J Nucl Cardiol 6:93–155
17. Thomas GS, Miyamoto MI, Morello AP 3rd et al (2004) Technetium 99m sestamibi myocardial perfusion imaging predicts clinical outcome in the community outpatient setting. The Nuclear Utility in the Community (NUC) Study. J Am Coll Cardiol 43:213-223
18. Giri S, Shaw LJ, Murthy DR et al (2002) Impact of diabetes on the risk stratification using stress single-photon emission computed tomography myocardial perfusion imaging in patients with symptoms suggestive of coronary artery disease. Circulation 105:32-40
19. Laing SP, Swerdlow AJ, Slater SD et al (2003) Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. Diabetologia 46:760-765
20. Jensen T, Borch-Johnsen K, Kofoed-Enevoldsen A, Deckert T (1987) Coronary heart disease in young type 1 (insulin-dependent) diabetic patients with and without diabetic nephropathy: incidence and risk factors. Diabetologia 30:144-148
21. Tuomilehto J, Borch-Johnsen K, Molarius A et al (1998) Incidence of cardiovascular disease in Type 1 (insulin-dependent) diabetic subjects with and without diabetic nephropathy in Finland. Diabetologia 41:784-790

22. Torffvit O, Lövestam-Adrian M, Agardh E, Agardh CD (2005) Nephropathy, but not retinopathy, is associated with the development of heart disease in Type 1 diabetes: a 12-year observation study of 462 patients. Diabet Med 22:723-729
23. Astrup AS, Tarnow L, Rossing P, Pietraszek L, Riis Hansen P, Parving HH (2005) Improved prognosis in type 1 diabetic patients with nephropathy: a prospective follow-up study. Kidney Int 68:1250-1257
24. Kim WY, Astrup AS, Stuber M et al (2007) Subclinical coronary and aortic atherosclerosis detected by magnetic resonance imaging in type 1 diabetes with and without diabetic nephropathy. Circulation 115:228-235
25. Watts GF, Powrie JK, O'Brien SF, Shaw KM (1996) Apolipoprotein B independently predicts progression of very-low-level albuminuria in insulin-dependent diabetes mellitus. Metabolism 45:1101-1107
26. O'Brien SF, Watts GF, Powrie JK, Shaw KM (1995) Exercise testing as a long-term predictor of the development of microalbuminuria in normoalbuminuric IDDM patients. Diabetes Care 18:1602-1605
27. Maahs DM, Rewers M (2006) Editorial: Mortality and renal disease in type 1 diabetes mellitus--progress made, more to be done. J Clin Endocrinol Metab 91:3757-3759
28. Schmermund A, Baumgart D, Görge G et al (1998) Measuring the effect of risk factors on coronary atherosclerosis: coronary calcium score versus angiographic disease severity. J Am Coll Cardiol 31:1267-1273
29. Olson JC, Edmundowicz D, Becker DJ, Kuller LH, Orchard TJ (2000) Coronary calcium in adults with type 1 diabetes: a stronger correlate of clinical coronary artery disease in men than in women. Diabetes 49:1571-1578
30. Anand DV, Lim E, Hopkins D et al (2006) Risk stratification in uncomplicated type 2 diabetes: prospective evaluation of the combined use of coronary artery calcium imaging and selective myocardial perfusion scintigraphy. Eur Heart J 27:713-721
31. Raggi P, Bellasi A, Ratti C (2005) Ischemia imaging and plaque imaging in diabetes: complementary tools to improve cardiovascular risk management. Diabetes Care 28:2787-2794
32. Scholte AJ, Schuijf JD, Kharagjitsingh AV et al (2008) Prevalence of coronary artery disease and plaque morphology assessed by multi-slice computed tomography coronary angiography and calcium scoring in asymptomatic patients with type 2 diabetes. Heart 94:290-295
33. Djaberi R, Schuijf JD, Boersma E et al (2009) Differences in atherosclerotic plaque burden and morphology between type 1 and 2 diabetes mellitus as assessed by multi-slice computed tomography. Diabetes Care 32:1507-1512

CHAPTER 11

Comparative Performance of Risk Stratification Tools for Predicting Functionally Relevant Coronary Artery Disease in Asymptomatic Type 2 Diabetes

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Submitted

ABSTRACT

Objective

Early identification of asymptomatic diabetic patients with coronary artery disease (CAD) has the potential to improve prognosis. For this purpose, selective screening algorithms that allow accurate identification of patients with a higher likelihood of functionally relevant CAD are needed. Several risk stratification tools have been suggested but no direct comparative data are available. The purpose of the present study was therefore to evaluate the comparative performance of Framingham risk score (FRS) and surrogate markers of CAD (carotid intima media thickness (CIMT), pulse wave velocity (PWV) and coronary artery calcium (CAC) scores) to identify functionally relevant coronary artery disease (CAD) in asymptomatic patients with type 2 diabetes.

Research design and methods

In 99 consecutive asymptomatic patients with type 2 diabetes, the FRS, CIMT, PWV and CAC were obtained. Patients were screened for the presence of functionally relevant CAD defined by the concurrent presence of significant coronary stenosis on CT angiography and abnormal myocardial perfusion on SPECT. The association between the FRS (low, intermediate, high), CIMT (≥75th percentile), PWV (quartiles) and CAC scoring (0, 1-100, 101-400, >400) with the presence of functionally relevant CAD was assessed.

Results

Functionally relevant CAD was observed in 24% of patients. FRS did not increase the AUC for predicting functionally relevant CAD (AUC 0.61, $P = 0.18$). In contrast, the AUC increased significantly for PWV (AUC 0.68, $P = 0.03$), CIMT (AUC 0.81, $P < 0.001$) and CAC scoring (AUC 0.84, $P < 0.001$). An excellent sensitivity and negative predictive value were observed with increased CIMT (83% and 95%) and a CAC score >100 (85% and 97%) for predicting functionally relevant CAD.

Conclusions

As compared to FRS and PWV, CIMT and CAC scoring were superior in predicting the presence of functionally relevant CAD. Accordingly, CIMT and CAC scoring may be preferred for identification of asymptomatic patients with type 2 diabetes requiring further testing.

INTRODUCTION

Type 2 diabetes presents a constellation of risk factors caused by insulin resistance resulting in elevated coronary artery disease (CAD) risk (1). Despite optimal medical treatment of cardiovascular risk factors, CAD related mortality remains high in this patient population (1). However, early identification of high-risk patients with extensive CAD that may benefit from aggressive intervention may improve outcome (2). The ADA/AHA have therefore previously recommended non-invasive screening for CAD by cardiac imaging techniques, in the presence of ≥ two additional cardiovascular risk factors (3). Nevertheless, this selection strategy was shown to be ineffective in the DIAD study, wherein 41% of asymptomatic diabetic patients with abnormal myocardial perfusion did not have ≥ two risk factors (4). Also, a wide ranged routine screening strategy of all diabetic patients by non-invasive cardiac imaging on the other hand was shown to be ineffective due to the low overall prevalence of abnormal myocardial perfusion in this trial (5). Possibly, a selective screening strategy, allowing identification of patients with a high likelihood of significant epicardial coronary stenosis resulting in abnormal myocardial perfusion (*functionally relevant* CAD) would be preferred and could improve clinical management (2). Indeed, knowledge of functionally relevant CAD has important implications for risk stratification and can guide clinical decision making regarding the need for revascularization (2,6).

Several risk stratification tools have been proposed as potential first-line screening methods in patients with type 2 diabetes (7-11). These approaches include assessment of the Framingham risk score (FRS) (7) or non-invasive surrogate markers as carotid intima media thickness (CIMT) (8-9), aortic pulse wave velocity (PWV) (10) and coronary artery calcium (CAC) scoring (9,11-12). However, the value of these approaches to identify *functionally relevant* CAD has not been extensively investigated in type 2 diabetic patients. In addition, direct comparisons are lacking.

The current study was therefore designed to prospectively evaluate the comparative performance of FRS, CIMT, PWV and CAC scoring to identify functionally relevant CAD (defined by the concurrent presence of significant coronary stenosis on CT angiography (CTA) and abnormal myocardial perfusion on SPECT) in asymptomatic patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Patients and study design

Prospectively, 99 consecutive asymptomatic patients with type 2 diabetes were screened for the presence of cardiovascular disease. All patients underwent a comprehensive evaluation, which included a structured interview, physical examination, blood serum

and urine laboratory testing to assess cardiovascular risk factors and determine FRS. CIMT, PWV and CAC scoring were obtained as surrogate markers of CAD. To directly determine the presence of functionally relevant CAD, combined anatomical and functional cardiac assessment was performed non-invasively using CTA and SPECT myocardial perfusion imaging.

Cardiovascular risk factors and FRS

Cardiovascular risk factors were assessed according to the following criteria: positive family history of CAD (presence of CAD in first degree family members male <55 years and/or female <65 years), smoking (current smoking or smoking in the last 2 years), body mass index (kg/m^2), hypertension (blood pressure >140/90 mmHg or treatment with antihypertensive medication), hypercholesterolemia (total cholesterol level >5.0 mmol/L or use of cholesterol lowering medication), plasma hemoglobin A1c and micro-albuminuria (urine albumin/creatinine ratio ≥3.5 mg/mmol).

The FRS adjusted for diabetes was calculated in all patients (7). Accordingly, patients were stratified as having a low (<10%), intermediate (10-20%) or high (>20%) 10-year risk for CAD.

Non-invasive measurement of surrogate markers of CAD and data analysis

PWV and CIMT were assessed by an experienced sonographer blinded to clinical information. Measurements were performed in fasting patients who had abstained from their morning medication, in a quiet, temperature controlled clinical research laboratory. PWV was determined using applanation tonometry (SphygmoCor, Atcor Medical, Sydney, Australia). Measurements started when a state of constant heart rate and blood pressure was reached after a 10 minute rest in supine position. The pulse waves were recorded at the common carotid artery and the femoral artery by sequential tonometry with simultaneous electrocardiographic gating. Pulse transit time was determined as the average of 10 consecutive beats. The distance between the two sites was measured. PWV (in m/s) was defined as the distance between the 2 recording sites traveled by the pulse wave, divided by transit time. Using system software, PWV was determined semi-automatically (13). Measurements were performed three times in each patient and averaged to obtain the mean PWV. Thereafter, patients were stratified according to mean PWV quartiles.

CIMT was assessed using high resolution B-mode ultrasound with a 10-MHz linear transducer, with an automatic boundary detection system (Art.Lab-Esaote-Picus, Genova, Italy). Mean CIMT was assessed throughout 10-mm segments, at four angles, across the far wall of the right and left common carotid artery. The average of the

mean CIMT values of the 4 segments was calculated to determine the mean right and left CIMT per patient. The obtained mean CIMT was compared to reference values from the MESA study (14). Patients were thereby stratified as having normal CIMT (CIMT <75th percentile), or increased CIMT (CIMT ≥75th percentile in at least one common carotid artery).

CAC scoring was performed with a 64-row CT scanner (Aquilion64, Toshiba Medical Systems, Japan), according to standard clinical protocols (15). The total Agatston score was determined for each patient. Patients were stratified according to the CAC score ranges of 0, 1-100, 101-400, and >400.

Assessment of functionally relevant CAD

CTA data acquisition and analysis

CTA was performed in the same session as CAC scoring, according to protocols described previously (15). CTA images were interpreted by two experienced observers. Discrepancies in interpretation were resolved by consensus. The presence of significant coronary stenosis, defined as the presence of luminal narrowing ≥50%, was visually evaluated on axial images and curved multiplanar reconstructions in at least two orthogonal planes.

SPECT data acquisition and analysis

Myocardial perfusion imaging was performed using ECG-gated SPECT with ^{99m}Tc sestamibi, after pharmacological stress, according to standard clinical protocols (16).

Short-axis slices were displayed in polar map format, adjusted for peak myocardial activity of 100%. Additional reconstruction yielded standard long- and short-axis projections perpendicular to the heart axis. All views were used for semi-quantitative interpretation. The myocardium was divided into 17 segments, and tracer uptake in each segment was evaluated by two observers in consensus using a 5-point scoring system (0:normal, 1:mild, 2:moderate, 3:severe reduction of tracer uptake and 4:apparent absence of tracer uptake) (17). The total segmental score during stress was used to determine the summed stress score (SSS) for each patient. The presence of abnormal myocardial perfusion was defined as SSS ≥3. Regional wall motion on gated SPECT images was analyzed to differentiate between true perfusion abnormalities and diaphragmatic or breast attenuation artifacts. Obvious or probable attenuation artifacts were interpreted as normal.

Finally, the presence of functionally relevant CAD was defined by the concurrent presence of significant coronary stenosis on CTA as well as abnormal myocardial perfusion.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation. Categorical variables were expressed as numbers (percentages).

First, patients were stratified according to the FRS category (low, intermediate and high). The prevalence of functionally relevant CAD was examined across the FRS risk score categories.

Thereafter, the potential of the FRS, PWV, CIMT, and CAC scoring for predicting the presence of functionally relevant CAD in asymptomatic patients with type 2 diabetes was compared. For this purpose, using C-statistics, the area under ROC curve (AUC) was calculated for FRS, PWV, CIMT, and CAC scoring. Finally, diagnostic accuracy was determined for the markers with the highest AUC. Statistical analyses were performed using SPSS software (version 16.0, Inc., Chicago, Illinois). *P* values <0.05 were considered statistically significant.

RESULTS

Patient characteristics

The study population comprised of 99 asymptomatic patients with type 2 diabetes. Baseline patient characteristics are provided in Table 1. At the time of referral, hypercholesterolemia was identified in 71% of the patients. Cholesterol lowering medication was used by 51% of the patients. The mean total cholesterol was 4.8 ± 1.2 mmol/L, the LDL-cholesterol 3.0 ± 1.1 mmol/L and the HDL-cholesterol 1.3 ± 0.4 mmol/L. Overall, 66% of the patients had hypertension, of which 51 patients (52% of total population) used anti-hypertensive medication. The mean systolic blood pressure was 134 ± 16 mmHg, and the mean diastolic pressure was calculated to be 82 ± 8 mmHg.

FRS assessment and surrogate markers of CAD

FRS: Calculation of the FRS for the 10-year risk of CAD in the study population resulted in classification of 42 patients (42%) at low risk, 37 (37%) at intermediate risk and 20 (21%) at high risk.

PWV: The average PWV was calculated to be 9.0 ± 2.0 m/s in the total population. The interquartile ranges were defined as <7.7 m/s, 7.7-9.3 m/s, 9.4-10.6 m/s and >10.6 m/s.

Table 1. Characteristics of the study population (n = 99)

Age (years)	54 ± 11
Men	51 (51%)
Diabetes duration (years)	9 ± 7
Smokers	22 (22%)
Family history of CAD	48 (49%)
BMI (kg/m^2)	30 ± 7
Hypercholesterolemia	70 (71%)
Hypertension	65 (66%)
HbA1c (mmol/L)	8.4 ± 1.9
Micro-albuminuria	30 (30%)
FRS distribution	
low risk	42 (42%)
intermediate risk	37 (37%)
high risk	20 (21%)

Data are averages ± standard deviation or number of patients (%). CAD: coronary artery disease; FRS: Framingham risk score.

CIMT: Mean CIMT was 0.68 ± 0.13 mm. Comparison with reference values revealed an increased CIMT value (≥75th percentile) in 38 patients (39%).
CAC scoring: Average CAC score was 203 ± 381 in the total population. CAC was absent in 45 patients (45%). The CAC score ranged 1-100 in 23 patients (23%), 101-400 in 13 patients (14%) and >400 in 18 (18%) of the asymptomatic patients with diabetes.

Presence of functionally relevant CAD

The presence of CAD was excluded on CTA in 64 patients (65%). In the remaining 35 patients (35%) significant coronary stenosis defined as luminal narrowing of ≥50% was observed. Using SPECT abnormal myocardial perfusion (SSS ≥3) was revealed in 34 patients (35%). Finally, in the total population of asymptomatic patients with diabetes, 23 patients (24%) were shown to have significant coronary stenosis on CTA accompanied by abnormal myocardial perfusion and were thus classified as having functionally relevant CAD.

Relation of FRS and the surrogate markers with functionally relevant CAD

The distribution of asymptomatic patients with diabetes with functionally relevant CAD according to the FRS category is provided in Figure 1. The proportion of patients with functionally relevant CAD increased gradually per risk level with the highest percentage observed in patients classified at high risk. Nevertheless, functionally relevant CAD was also observed in 13% of patients classified at low risk.

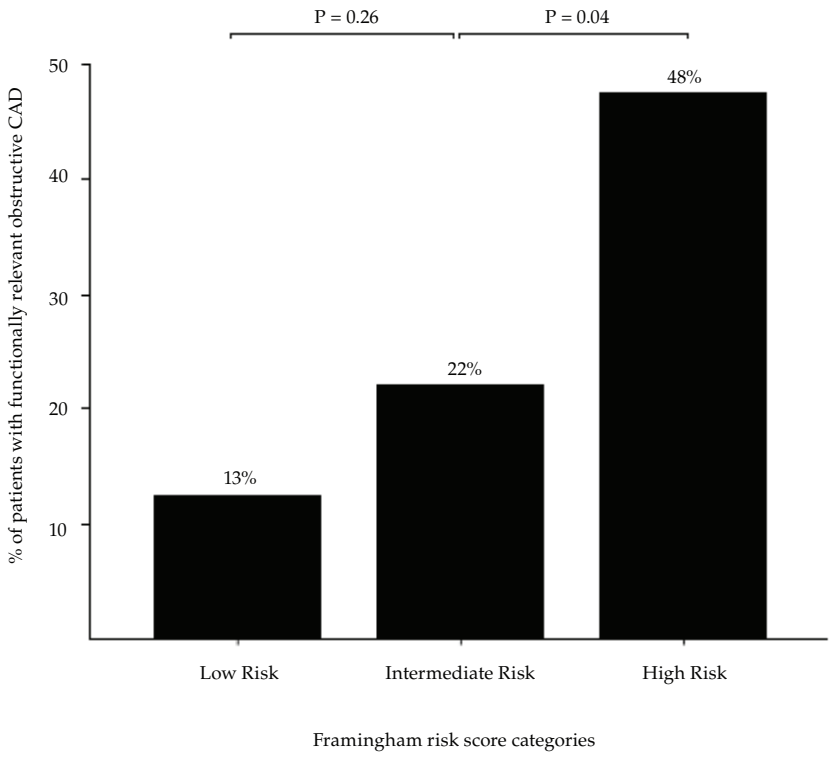
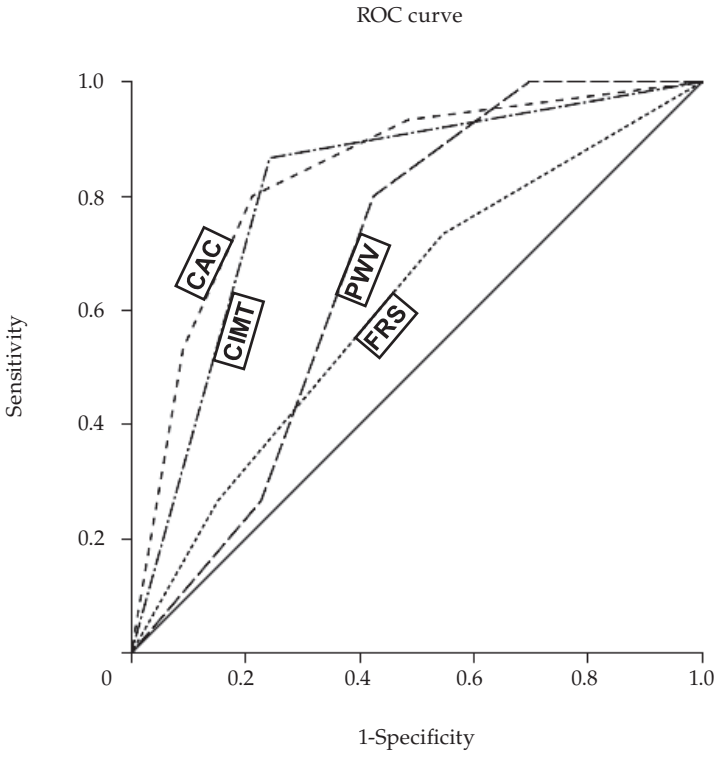


Figure 1. Distribution of asymptomatic patients with type 2 diabetes with functionally relevant CAD according to the Framingham risk score category. The proportion of patients with functionally relevant CAD increased gradually per risk level. However, functionally relevant CAD was present in still 13% of patients stratified as having low risk.



Parameter	AUC	95% CI	P-value
FRS (per category: low, intermediate, high)	0.61	(0.45 -0.77)	0.18
PWV (per quartile)	0.68	(0.56 -0.80)	0.03
Increased CIMT (left or right)	0.81	(0.69 -0.93)	<0.001
CAC score (per range: 0, 1 - 100, 101 - 400, >400)	0.84	(0.73 -0.95)	<0.001

Figure 2. ROC curves and corresponding C-statistics data for FRS, PWV, CIMT and CAC scoring to identify functionally relevant CAD in asymptomatic patients with type 2 diabetes. PWV, CIMT and CAC scoring significantly increased the AUC. Notably, the highest AUC was observed for CIMT and CAC scoring.

The data for C-statistics for the AUC and the corresponding ROC curves for the FRS, PWV, CIMT and CAC for predicting the presence of functionally relevant CAD are provided in Figure 2. FRS did not significantly increase the AUC (AUC 0.61, $P = 0.18$). However, a significant increase in AUC was observed for all surrogate markers of CAD. Although significant, the increase in AUC was limited using PWV (AUC 0.68, $P = 0.03$). Of note, the AUC was high for CIMT (AUC 0.81, $P < 0.001$). Similarly, a good AUC was obtained for CAC scoring (AUC 0.84, $P < 0.001$).
Further ROC curve analysis for CIMT resulted in a sensitivity of 87%, specificity of 76%, negative predictive value of 95% and a positive predictive value of 48% for predicting the presence of functionally relevant CAD. Similarly, a CAC score >100 yielded a sensitivity of 85%, specificity of 83%, negative predictive value of 97% and a positive predictive value of 46% for predicting the presence of functionally relevant CAD. Using a higher threshold for CAC (score >400), a lower sensitivity of 60% and a specificity of 81%, with a negative predictive value of 93% and a positive predictive value of 63% were found.

CONCLUSIONS

In asymptomatic patients with type 2 diabetes, functionally relevant CAD was observed in 24% of patients. When evaluating the relation between increasing FRS and the presence of functionally relevant CAD, a gradual increase was observed from low to high risk. However, functionally relevant CAD was still observed in a substantial number of asymptomatic patients stratified as having low risk (13%). Moreover, FRS did not increase the AUC for predicting functionally relevant CAD. In contrast, a better performance in the prediction of the presence of functionally relevant CAD was observed for the surrogate markers of CAD. Particularly, a good AUC was observed for CIMT (AUC 0.81) and CAC scoring (AUC 0.84). In continuation of these findings, excellent sensitivities and negative predictive values were observed using CIMT (83% and 95%) and a CAC score >100 (85% and 97%) for predicting functionally relevant CAD in asymptomatic patients with type 2 diabetes.

Risk stratification in asymptomatic patients with type 2 diabetes

To improve prognosis, screening of asymptomatic patients with type 2 diabetes for CAD has been proposed to facilitate early identification and patient-tailored treatment. A variety of first-line risk stratification methods have been proposed as an initial step for selective screening of asymptomatic patients with type 2 diabetes (2,3, 6-12). The aim of these algorithms is to identify within the asymptomatic diabetic population a subgroup that is at higher risk and that may benefit from further imaging potentially followed by revascularization.

Refined risk calculators, especially the FRS, have been well validated for CAD risk assessment in the general population (18). However, the general FRS equation incorporates diabetes as a categorical variable and is shown to underestimate the risk of CAD events in diabetic populations (18). In the current study, the modified FRS equation by Wilson et al was applied to predict the presence of functionally relevant CAD in patients with type 2 diabetes (7). While a significant relation between FRS and the prevalence of functionally relevant CAD was observed, still a non-negligible portion (13%) of patients at low risk were shown to have functionally relevant CAD. Possibly, surrogate markers, as estimates of atherosclerosis, may present a more suitable approach to identify CAD as compared to risk engines.

For this purpose, several tools are available that may provide a practical first-line approach for selective screening. PWV, a marker of vascular stiffness, has been shown to increase with the presence and severity of CAD both in the general population and in patients with type 2 diabetes (10, 19). More information is available on CIMT, which has been shown to be a consistent predictor of CAD in large prospective epidemiological studies (20). Also in type 2 diabetes, the relation between CIMT and CAD has been confirmed in sub-analyses of the ARIC and the IRAS investigations (21-22). Finally, extensive data have been obtained with CAC, supporting its potential use in selective screening algorithms (23). Comparative data however, are scarce and predominantly lacking in patients with diabetes.

In line with previous investigations, PWV was shown to improve prediction of functionally relevant CAD as compared to risk stratification based on FRS. As expected however, AUC to predict functionally relevant CAD was lower for PWV as compared to the more direct measures of atherosclerosis provided by CIMT and CAC. Aortic stiffness is regulated by numerous factors and in turn contributes to atherosclerosis and reduced myocardial perfusion by several separate mechanisms (24). Therefore, PWV should preferably be considered as a marker of *total* cardiovascular risk rather than specifically of atherosclerotic changes alone. More accurate prediction of functionally relevant CAD may be achieved through direct visualization of atherosclerosis. Indeed, in the current study, CIMT (AUC 0.81) and CAC scoring (AUC 0.84) were superior to PWV (AUC 0.68) for the identification of functionally relevant CAD. In a next step, diagnostic accuracy for increased CIMT and CAC were explored. Using a CIMT value $\geq 75^{th}$ percentile to define increased CIMT, as recommended by the Society for Vascular Medicine consensus (14), a good sensitivity of 87% and high negative predictive value of 95% to identify functionally relevant CAD were obtained. Similarly, a CAC score >100 yielded a sensitivity of 85% with a corresponding negative predictive value of 97%. Interestingly, changing the cut-off value to define increased CAC from 100 to 400, which is commonly used in the general population, resulted in lower accuracy. This is in fact

in line with previous literature, suggesting that in the presence of diabetes, lower CAC thresholds should be used (23,25-27).
In addition to diagnostic performance however, also other criteria, such as safety, reproducibility, costs and practicality, should be taken into account when determining the most appropriate first-line screening tool. While PWV may have the advantage of lowest costs as well as simplicity, disadvantages include lower accuracy and to a lesser extent, lower reproducibility (28). For both CIMT and CAC, accuracy and reproducibility are higher, while still being relatively straightforward techniques. For CAC however, costs are highest while also the associated radiation dose remains a limitation (29). On the other hand, CAC may have the advantage of highest accuracy as well as strong prognostic value for future cardiovascular events (23). Further investigations should address these issues into more detail, while also providing comparative prognostic data.

In summary, the surrogate markers of CAD, including PWV, CIMT and CAC scoring, but not FRS, were shown to be significantly associated with the presence of functionally relevant CAD in asymptomatic patients with type 2 diabetes. An increased CIMT measurement and a CAC score of >100 were revealed superior in predicting the presence of functionally relevant CAD with good sensitivity and high negative predictive value. Accordingly, CIMT assessment and CAC scoring convey the most potential to identify asymptomatic patients with type 2 diabetes at highest risk for functionally relevant CAD.

REFERENCES

1. Vamos EP, Millett C, Parsons C, Aylin P, Majeed A, Bottle A. Nationwide study on trends in hospital admissions for major cardiovascular events and procedures among people with and without diabetes in England, 2004-2009. Diabetes Care 2012;35:265-272
2. Brooks MM, Chaitman BR, Nesto RW, Hardison RM, Feit F, Gersh BJ, Krone RJ, Sako EY, Rogers WJ, Garber AJ, King SB 3rd, Davidson CJ, Ikeno F, Frye RL; BARI 2D Study Group. Clinical and angiographic risk stratification and differential impact on treatment outcomes in the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial. Circulation 2012;126:2115-2124
3. Consensus development conference on the diagnosis of coronary heart disease in people with diabetes: 10-11 February 1998, Miami, Florida. American Diabetes Association. Diabetes Care 1998;21:1551-1559
4. Wackers FJ, Young LH, Inzucchi SE, Chyun DA, Davey JA, Barrett EJ, Taillefer R, Wittlin SD, Heller GV, Filipchuk N, Engel S, Ratner RE, Iskandrian AE; Detection of Ischemia in Asymptomatic Diabetics Investigators. Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. Diabetes Care 2004; 27:1954-1961
5. Young LH, Wackers FJ, Chyun DA, Davey JA, Barrett EJ, Taillefer R, Heller GV, Iskandrian AE, Wittlin SD, Filipchuk N, Ratner RE, Inzucchi SE; DIAD Investigators. Cardiac outcomes after screening for asymptomatic coronary artery disease in patients with type 2 diabetes: the DIAD study: a randomized controlled trial. JAMA 2009;301:1547-1555
6. Momose M, Nakajima K, Nishimura T. Prognostic significance of stress myocardial gated SPECT among Japanese patients referred for coronary angiography: A study of data from the J-ACCESS database. Eur J Nucl Med Mol Imaging 2009;36:1329-1337
7. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB.cardial gated SPECT among Japanese patients referred for coronary angiography: A study of data from the J-ACCESS database. Prediction of coronary heart disease using risk factor categories. Circulation 1998;97:1837-1847
8. Bernard S, Sérusclat A, Targe F, Charrière S, Roth O, Beaune J, Berthezène F, Moulin P. Incremental predictive value of carotid ultrasonography in the assessment of coronary risk in a cohort of asymptomatic type 2 diabetic subjects. Diabetes Care 2005;28:1158-1162
9. Raggi P, Bellasi A, Ratti C. Ischemia imaging and plaque imaging in diabetes: complementary tools to improve cardiovascular risk management. Diabetes Care 2005;28:2787-2794
10. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation 2002;106:2085-2090

11. Elkeles RS, Godsland IF, Feher MD, Rubens MB, Roughton M, Nugara F, Humphries SE, Richmond W, Flather MD; PREDICT Study Group. Coronary calcium measurement improves prediction of cardiovascular events in asymptomatic patients with type 2 diabetes: the PREDICT study. Eur Heart J 2008;29:2244-2251
12. Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ, Fayad ZA, Foster E, Hlatky MA, Hodgson JM, Kushner FG, Lauer MS, Shaw LJ, Smith SC Jr, Taylor AJ, Weintraub WS, Wenger NK, Jacobs AK; American College of Cardiology Foundation/American Heart Asscoiation Tasl Force on Practice Guidelines. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation 2010;122:2748-2764
13. Wilkinson IB, Fuchs SA, Jansen IM, Spratt JC, Murray GD, Cockcroft JR, Webb DJ. Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. J Hypertens 1998;16:2079-2084
14. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, Najjar SS, Rembold CM, Post WS; American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 2008;21:93-111
15. Djaberi R, Schuijf JD, Boersma E, Kroft LJ, Pereira AM, Romijn JA, Scholte AJ, Jukema JW, Bax JJ. Differences in atherosclerotic plaque burden and morphology between type 1 and 2 diabetes as assessed by multislice computed tomography. Diabetes Care 2009;32:1507-1512
16. Scholte AJ, Schuijf JD, Kharagjitsingh AV, Dibbets-Schneider P, Stokkel MP, van der Wall EE, Bax JJ. Prevalence and predictors of an abnormal stress myocardial perfusion study in asymptomatic patients with type 2 diabetes mellitus. Eur J Nucl Med Mol Imaging 2009;36:567-575
17. Hansen CL, Goldstein RA, Akinboboye OO, Berman DS, Botvinick EH, Churchwell KB, Cooke CD, Corbett JR, Cullom SJ, Dahlberg ST, Druz RS, Ficaro EP, Galt JR, Garg RK, Germano G, Heller GV, Henzlova MJ, Hyun MC, Johnson LL, Mann A, McCallister BD Jr, Quaife RA, Ruddy TD, Sundaram SN, Taillefer R, Ward RP, Mahmarian JJ; American Society of Nuclear Cardiology. Myocardial perfusion and function: single photon emission computed tomography. J Nucl Cardiol 2007;14:e39-60
18. Coleman RL, Stevens RJ, Retnakaran R, Holman RR. Framingham, SCORE, and DECODE risk equations do not provide reliable cardiovascular risk estimates in type 2 diabetes. Diabetes Care 2007;30:1292-1293

19. Roos CJ, Djaberi R, Schuijf JD, de Koning EJ, Rabelink TJ, Smit JW, Pereira AM, Al Younis I, van der Hiel B, Scholte AJ, Bax JJ, Jukema JW. Relationship between vascular stiffness and stress myocardial perfusion imaging in asymptomatic patients with diabetes. Eur J Nucl Med Mol Imaging 2011;38:2050-2057
20. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation 2007;115:459-467
21. Haffner SM, Agostino RD Jr, Saad MF, O'Leary DH, Savage PJ, Rewers M, Selby J, Bergman RN, Mykkänen L. Carotid artery atherosclerosis in type-2 diabetic and nondiabetic subjects with and without symptomatic coronary artery disease (The Insulin Resistance Atherosclerosis Study). Am J Cardiol 2000;85:1395-1400
22. Lee CD, Folsom AR, Pankow JS, Brancati FL; Atherosclerosis Risk in Communities (ARIC) Study Investigators. Cardiovascular events in diabetic and nondiabetic adults with or without history of myocardial infarction. Circulation 2004;109:855-860
23. Agarwal S, Cox AJ, Herrington DM, Jorgensen NW, Xu J, Freedman BI, Carr JJ, Bowden DW. Coronary calcium score predicts cardiovascular mortality in diabetes: diabetes heart study. Diabetes Care 2013;36:972-977
24. London GM, Marchais SJ, Guerin AP, Pannier B. Arterial stiffness: pathophysiology and clinical impact. Clin Exp Hypertens 2004;26:689-99
25. Anand DV, Lim E, Hopkins D, Corder R, Shaw LJ, Sharp P, Lipkin D, Lahiri A. Risk stratification in uncomplicated type 2 diabetes: prospective evaluation of the combined use of coronary artery calcium imaging and selective myocardial perfusion scintigraphy. Eur Heart J 2006;27:713-721
26. Church TS, Levine BD, McGuire DK, Lamonte MJ, Fitzgerald SJ, Cheng YJ, Kimball TE, Blair SN, Gibbons LW, Nichaman MZ. Coronary artery calcium score, risk factors, and incident coronary heart disease events. Atherosclerosis 2007;190:224-231
27. Raggi P, Shaw LJ, Berman DS, Callister TQ. Prognostic value of coronary artery calcium screening in subjects with and without diabetes. J Am Coll Cardiol 2004;43:1663-1669
28. Simon A, Chironi G, Levenson J. Comparative performance of subclinical atherosclerosis tests in predicting coronary heart disease in asymptomatic individuals. Eur Heart J 2007;28:2967-2971
29. McCollough CH, Ulzheimer S, Halliburton SS, Shanneik K, White RD, Kalender WA. Coronary artery calcium: a multi-institutional, multimanufacturer international standard for quantification at cardiac CT. Radiology 2007;243:527-538

PART III

Microvascular disease

CHAPTER 12

Non-Invasive Assessment of Microcirculation by Sidestream Dark Field Imaging as a Marker of Coronary Artery Disease in Diabetes

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ABSTRACT

Purpose

In diabetes, generalized microvascular disease and coronary artery disease (CAD) are likely to occur in parallel. We used sidestream-dark-field (SDF) handheld imaging device to determine the relation between the labial microcirculation parameters and CAD in asymptomatic patients with diabetes.

Methods

SDF imaging was validated for assessment of labial capillary density and tortuosity. Thereafter, mean labial capillary density and tortuosity were evaluated and compared in non-diabetic controls, and in asymptomatic patients with type 1- and type 2 diabetes. In diabetic patients, mean capillary density and tortuosity were compared according to the presence of CAD.

Results

Both type 1- and type 2 diabetes were associated with increased capillary density and tortuosity. In diabetes, mean capillary density was an independent predictor of elevated CAC ($P = 0.03$) and obstructive CAD on CT-angiography ($P = 0.01$). Using a cut-off mean capillary density of 24.9 (per 0.63 mm^2) the negative predictive value was 84% and 89% for elevated CAC and obstructive CAD. Likewise, capillary tortuosity was an independent predictor of increased CAC ($P = 0.01$) and obstructive CAD ($P = 0.04$).

Conclusion

Assessment of labial microcirculation parameters using SDF imaging is feasible and conveys the potential to estimate vascular morbidity in patients with diabetes, at bedside.

INTRODUCTION

Cardiovascular disease, especially coronary artery disease (CAD), is a predominant cause of morbidity and mortality in diabetes.[1] As a result, recent research has aimed to determine additional risk factors and markers, to distinguish high risk diabetic patients.[2,3] Likewise, the presence of microvascular co-morbidities, in form of nephropathy, retinopathy and neuropathy, has been previously associated with an increased risk of CAD as well as its worse prognosis in diabetes.[4-7] Generalized microvascular disease and CAD may occur in parallel due to common pathogenic mechanisms initiated by hyperglycaemia.[8] However, microvascular disease has also been suggested to contribute to CAD directly through angiogenesis of microvessels in the atherosclerotic plaque.[9] As a consequence, a measure to quantify and qualify microvascular disease in diabetes may convey the potential to predict vascular morbidity and CAD more accurately than the traditional risk factors.

The orthogonal polarization spectral (OPS) and the more novel sidestream dark field (SDF) handheld imaging device allow direct visualization of blood in the microcirculation.[10,11] Thereby, the microcirculatory network of arterioles and capillaries can be investigated non-invasively. In particular, the technique is suitable for the study of easily accessible tissues with a superficial microcirculatory network of the skin and mucous membranes. Accordingly, OPS and SDF imaging have been applied to assess the characteristics of the microcirculation and monitor its alterations in the nail fold as well as in sublingual and labial tissue of patients with heart failure, rheumatic diseases and sepsis.[12,13] However, to our knowledge no previous studies have been performed in patients with diabetes.

In the current study we first sought to validate the assessment of labial microcirculation parameters, comprising of capillary density and tortuosity, using the SDF imaging device. Secondly, the labial capillary density and tortuosity were compared in non-diabetic controls and patients with diabetes. Finally, the relation between labial capillary density and tortuosity with CAD was evaluated in the sub-population of patients with diabetes.

METHODS

Study design and population

One hundred and thirty-one consecutive asymptomatic patients with diabetes were referred to the cardiology outpatient clinic for cardiovascular screening. The American Diabetes Association criteria were used to define diabetes and for further stratification in type 1 or 2.[14] Patients were considered as having type 1 diabetes if laboratory analysis demonstrated auto-antibodies to islet cells, insulin and glutamic acid decarboxylase. Otherwise, patients were considered to have type 2 diabetes. Further cardiovascular risk

factors were assessed according to the following criteria: positive family history of CAD (defined as presence of CAD in first degree family members younger than 55 (men) or 65 (women) years of age), smoking (defined as current smoking or smoking in the last 2 years), hypertension (defined as a blood pressure >140/90 mmHg or treatment with antihypertensive medication), hypercholesterolemia (defined as a total cholesterol level >5.0 mmol/L or use of cholesterol lowering medication), degree of obesity (estimated by body mass index [BMI = Kg/m^2]), level of glycemic control defined by plasma glycated-haemoglobin (mmol/L) and presence of micro-albuminuria (defined by a urine albumin/creatinine ratio ≥3.5 mg/mmol). Second, non-invasive multi-slice CT (MSCT), including coronary artery calcium (CAC) scoring and coronary angiography, were performed as part of clinical work up. Also, all patients underwent non-invasive assessment of the labial microcirculation using SDF imaging, to determine capillary density and tortuosity. The latter was performed in a study setting, performed according to the Declaration of Helsinki and approved by the institutional review committee of the Leiden University Medical Centre, Leiden. All patients gave written informed consent.
In addition, as part of the study setting, 50 asymptomatic healthy individuals comprising the non-diabetic control group, underwent a similar non-invasive assessment of the labial microcirculation using SDF. The healthy individuals comprising this control group had no history of diabetes or cardiovascular disease and were not known with related risk factors (hypertension, hypercholesterolemia, smoking or micro-albuminuria).

Validation study of the microcirculation parameters as assessed by SDF
The intra- and interobserver variability of the labial capillary density and structure was determined in the non-diabetic control group (n = 50). For this sub-population, SDF imaging of the four inner lip quadrants was performed by two experienced observers. SDF imaging was performed twice by each observer, on two different occasions. Each observer independently performed processing of own recordings followed by assessment of the capillary density and tortuosity.

Assessment of labial microcirculation

Data acquisition by Side-stream Dark Field imaging
Imaging of the capillaries was performed with SDF imaging with a handheld MicroScan Video Microscope (MicroVision Medical, Amsterdam, Netherlands). The SDF device was fitted with a sterile disposable 5x magnification lens. Video output was visualized on a monitor and connected to a computer via a signal converter (Canopus, ADVC110).
Measurements were performed by two trained physicians blinded to clinical data. All subjects (patients with diabetes and non-diabetic controls) were instructed to refrain

from caffeine containing substances 2 h prior to the evaluation. Subjects were in supine position, in a temperature controlled room with a temperature of approximately 22ºC. The tip of the SDF probe was placed on the inner lip. To prevent microcirculatory perfusion disturbance due to application of pressure on the imaging area, the probe was first placed on the labial tissue and then retracted to an extent which minimized contact but enabled visualization of the capillary bed. Illumination intensity and depth of focus were modulated to fine-tune image quality.

Continuous digital image recordings (duration 1 minute) were captured in four quadrants of the inner lip: upper right quadrant, upper left quadrant, lower right quadrant and lower left quadrant. Per quadrant, digital image recordings were saved on a hard drive as DV-AVI files to enable off-line analysis.

Assessment of microcirculation

For further assessment of capillary density and structure, 3 frozen microcirculatory imaging areas were selected from the digital image recordings for each quadrant. Microcirculatory imaging areas were selected to meet the following criteria: 1) representative capillary density and structure for the studied quadrant, 2) longitudinal axis view with full-length capillaries enabling structural as well as quantitative assessment of the capillaries, 3) clear, well-focused view of the capillaries. Each microcirculatory imaging area visualized by SDF corresponded with a tissue area of 0.63 mm^2 (0.9 mm x 0.7 mm) (Figure 1).

Capillary density

To determine capillary density, the number of capillaries was counted manually on each selected microcirculatory imaging area, on the monitor. All vessels identified as capillaries were included. Partially visible capillaries were included if the observer was certain that the vessel was a capillary due to its morphology. Capillary density was defined as the number of counted capillaries per microcirculatory image area (capillaries per 0.63 mm^2) (Figure 1). Finally, capillary density of the 12 microcirculatory imaging areas (3 microcirculatory imaging areas per quadrant) were averaged to obtain the mean capillary density per subject.

Capillary tortuosity

To assess the capillary tortuosity score, the number of twists per capillary in the majority of capillaries was evaluated, on each selected microcirculatory imaging area. The number of twists was stratified as 0: no twists (or pinhead capillaries) *to* 4: 4 or more twists (Figure 1). Subsequently, the overall tortuosity score per subject was determined

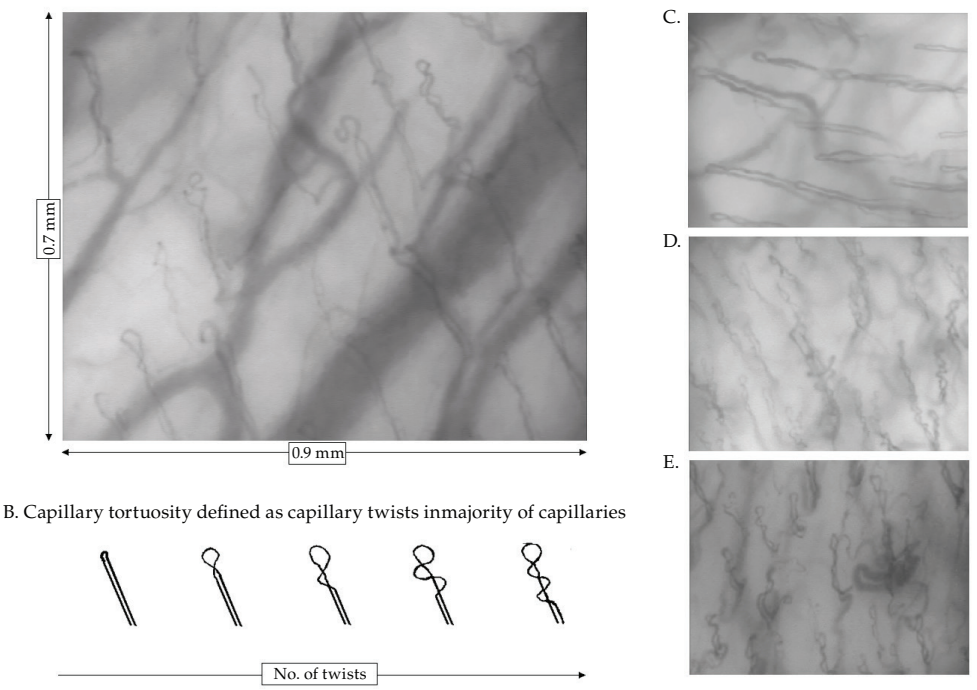

Figure 1. Visualization of the labial micro-vasculature by SDF. Capillaries are identified as loops emerging from the wider arterioles in the background (1A, 1C-E). To assess capillary density the number of capillaries was determined in a visual field of 0.63 mm^2 (1A). To assess capillary tortuosity, the number of twists per capillary in the majority of capillaries, was evaluated for each patient. Number of twists was stratified as 0: no twists (or pinhead capillaries) *to* 4: 4 or more twists (1B). A relatively low capillary density and tortuosity score was observed in non-diabetic controls (1C). In contrast, a higher capillary density and tortuosity score was observed in patients with diabetes (1D), often accompanied by dilation, branching and malformation of the capillaries (1E).

by selecting the most frequent tortuosity score in the 12 studied microcirculatory imaging areas.

Assessment of coronary artery disease by MSCT in patients with diabetes

MSCT data acquisition

Imaging was performed with a 64-slice MSCT scanner (Aquilion64, Toshiba Medical Systems, Japan). If necessary and tolerated, oral beta-blockers (metoprolol 50 mg or

100 mg) were provided 1 hour preceding the scan to achieve a heart rate <65 beats per minute. Initially, a non-enhanced prospective electrocardiographically gated scan, triggered at 75% of the R-R interval with 4 x 3.0 mm collimation was obtained to measure CAC score and determine the start and end position of the helical scan.
Thereafter, MSCT angiography was performed using the following parameters: collimation 64 x 0.5 mm, tube rotation time 400, 450 or 500 ms depending on the heart rate, tube current 300 or 350 mA, tube voltage 120 kV. Non-ionic contrast material was administered in the antecubital vein at a flow rate of 5 ml/L and the amount of 90–105 ml (depending on the total scan time), followed by 50 ml of saline solution flush. Automated bolus-tracking in the aortic root was used for the timing of the scan. Images were acquired with simultaneous ECG registration during a single breath hold of approximately 10 seconds. Segmental reconstruction algorithm was applied to generate a single image from the data of one, two or three consecutive heartbeats. Images were reconstructed in the cardiac phase showing least motion artifacts. In general, the end-diastolic phase was used. However, additional reconstructions were made throughout the entire cardiac cycle if necessary to improve image quality. Subsequently, the images were transferred to a remote workstation (Vitrea 2, Vital Images, Minnetonka, USA) for post-processing.

Assessment of CAD

Coronary artery calcium score
All data were evaluated with a remote workstation using dedicated software (Vitrea2, Vital Images, Minnetonka, USA). In each patient, coronary calcium was identified as a dense area in the coronary artery exceeding the threshold of 130 Hounsfield units. The total Agatston score was determined for each patient. Patients with a CAC score >100 were classified as having increased CAC.

Coronary atherosclerosis
MSCT coronary angiography images were interpreted by two experienced observers blinded to the patient characteristics. Discrepancies in interpretation were directly resolved in consensus. The presence of coronary atherosclerosis was visually evaluated on axial images and curved multiplanar reconstructions in at least two orthogonal planes. Obstructive coronary atherosclerosis was defined as the presence of luminal narrowing ≥50%.

Statistical analysis

Continuous variables were expressed as means ± standard deviation. Categorical variables were expressed as numbers (percentages).

First, for the validation of the mean labial capillary density assessment in non-diabetic controls, the interobserver for the first and second session, and the intraobserver for observer-1 as well as observer-2, were determined by calculating the Pearson coefficient of correlation (*r*). For the validation of the capillary tortuosity assessment in the control group, the interobserver for the first and second session, and the intraobserver for observer-1 as well as observer-2, were determined by calculating the agreement percentage and the *kappa* value.

Second, the mean capillary density was compared in non-diabetic controls and patients with type 1- and type 2 diabetes. For this purpose, the average capillary density and standard deviation were calculated in each group. The independent T-test was used to assess the difference in mean capillary density between the three groups. In addition, the relation between type 1 diabetes (versus non-diabetic controls as reference) and type 2 diabetes (versus non-diabetic controls as reference) with the capillary density was tested in a backward multivariate linear regression analysis, to correct for the influence of other cardiovascular risk factors.

Third, the distribution of capillary tortuosity was compared among healthy individuals, patients with type 1- and type 2 diabetes, by calculating the percentage of patients per tortuosity score for each group. Subsequently, the relation between type 1 diabetes (versus non-diabetic controls as reference) and type 2 diabetes (versus non-diabetic controls as reference) was also tested with the capillary tortuosity in a backward multivariate linear regression analysis.

Finally, in the sub-population of patients with diabetes, the relation of capillary density and tortuosity score with the presence of CAD was evaluated. Initially, patients with diabetes were stratified as having a CAC score 0-100 or as those with an elevated CAC score of >100. Average capillary density and standard deviation were calculated in each group. The independent T-test was used to assess the difference in mean capillary density between the two groups. A similar procedure was performed to compare the mean capillary density between diabetic patients with no obstructive CAD and those with obstructive CAD (luminal narrowing ≥50%).

To identify the potential predictors of an elevated CAC score in diabetes, a univariate logistic regression analysis of baseline cardiovascular risk factors, capillary density and capillary tortuosity was initially performed. Thereafter, all risk factors as well as capillary density and tortuosity were entered in a backward stepwise multiple logistic regression analysis model to identify the independent predictors of an elevated CAC

Table 1. Characteristics of non-diabetic controls and patients with type 1- and type 2 diabetes.

	Non–Diabetic Controls N = 50	Type 1 Diabetes N = 61	P-value*	Type 2 Diabetes N = 70	P-value**
Age	57 ± 14	46 ± 12	<0.001	55 ± 12	0.62
Male sex	27 (53%)	37 (61%)	0.43	33 (47%)	0.53
Diabetes duration (years)	-	24 ± 14	-	10 ± 7	-
Family history of CAD	11 (22%)	28 (46%)	0.01	34 (49%)	0.004
Smoking	0 (0%)	20 (33%)	<0.001	14 (20%)	<0.001
Body mass index (kg/m^2)	24 ± 3	25 ± 4	0.44	30 ± 7	<0.001
HbA1c (mmol/L)	-	7.8 ± 1.5	-	8.3 ± 1.6	-
Micro-albuminuria	-	9 (13%)	-	18 (26%)	-
Hypercholesterolemia	-	36 (59%)	-	50 (71%)	-
Hypertension	-	29 (48%)	-	46 (66%)	-
Statin use	0 (0%)	22 (36%)	<0.001	38 (54%)	<0.001
Anti-hypertensive medication	0 (0%)	19 (31%)	<0.001	41 (59%)	<0.001

* Difference in distribution between non-diabetic controls and patients with type 1 diabetes.

** Difference in distribution between non-diabetic controls and patients with type 2 diabetes. CAD = Coronary artery disease; HbA1c = glycated haemoglobin;

score. Results of variables with a P value ≤0.25 are illustrated. A similar procedure was repeated to identify the predictors of obstructive CAD in patients with diabetes.
Statistical analyses were performed using SPSS software (version 12.0.1, Inc., Chicago, Illinois). *P* values <0.05 were considered statistically significant.

RESULTS

Characteristics of non-diabetic controls and patients with diabetes

Characteristics of the sub-populations of non-diabetic controls, patients with type 1- and type 2 diabetes are provided in Table 1. No significant difference was observed among the gender distribution of patients with diabetes (type 1 and type 2) as compared to non-diabetic controls. Patients with type 1 diabetes were relatively younger than non-diabetic controls. In contrast, patients with type 2 diabetes were similar in age to non-diabetic controls. Patients with type 2 diabetes had a higher mean BMI than non-diabetic controls. As compared to non-diabetic controls, patients with diabetes (type 1 and type 2) were more often smokers, more often had a positive family history for CAD, and were frequently treated with statins and anti-hypertensive medication.

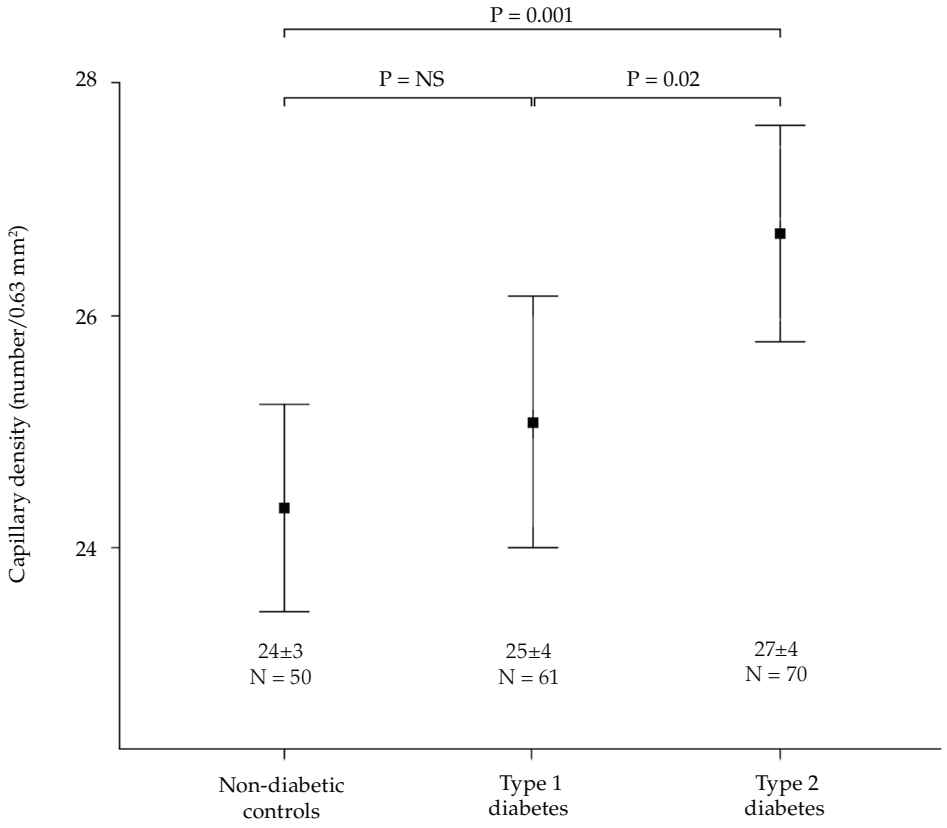

Figure 2. Mean capillary density in non-diabetic controls, versus type 1 diabetic patients, and type 2 diabetic patients. Mean capillary density was significantly higher in patients with type 2 diabetes.

Validation study

Capillary density

The interobserver correlation for the assessment of capillary density during the first session and second session were reasonable with a regression coefficient of 0.75 (P <0.001) and 0.72 (P <0.001) respectively. The intraobserver regression correlation coefficients for the assessment of capillary density were 0.80 (P <0.001) and 0.72 (P <0.001) for observer-1 and observer-2.

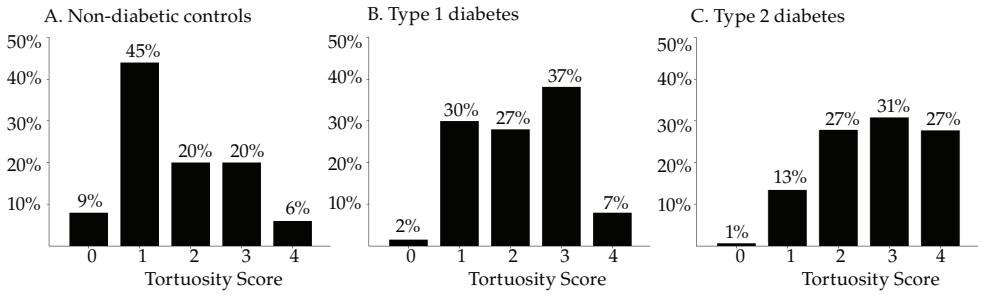

Figure 3. Distribution of patients according to capillary tortuosity score. A lower tortuosity score was observed in non-diabetic controls (3A). In type 1 diabetes, patients were rather evenly distributed among a tortuosity score of 1-3 (3B). In comparison, in type 2 diabetes a larger proportion of patients were shown to have a relatively high tortuosity score (3C).

Capillary tortuosity

The interobserver values for the assessment of capillary tortuosity were excellent and similar for the first and second session with an agreement of 88% in tortuosity score (*kappa* 0.83). The intraobserver evaluation for tortuosity score revealed an agreement of 90% (*kappa* 0.85) for observer-1 and 88% (*kappa* 0.83) for observer-2.

Labial capillary density in patients with diabetes as compared to non-diabetic controls

Capillary density in type 1 diabetes

The mean capillary density in patients with type 1 diabetes (25 ± 4 per 0.63 mm^2) was not significantly different as compared to non-diabetic controls (24 ± 3 per 0.63 mm^2) (Figure 2). However, after correction for age and other cardiovascular risk factors in a backward multiple linear regression model, the presence of type 1 diabetes (with non-diabetic controls as reference) was found to be associated with an increased capillary density (Exp ß 2.4, 95% CI 0.8-3.9; $P = 0.003$).

Capillary density in type 2 diabetes

The mean capillary density was significantly increased in patients with type 2 diabetes (27 ± 4 per 0.63 mm^2) as compared to non-diabetic controls ($P = 0.001$) (Figure 2). Importantly, also after correction for age and other cardiovascular risk factors in a backward multiple linear regression model, the presence of type 2 diabetes (with non-diabetic controls as reference) maintained a significant association with capillary density (Exp ß 1.0, 95% CI 0.3-1.6; $P = 0.01$).

Labial capillary tortuosity in patients with diabetes as compared to non-diabetic controls

Capillary tortuosity in type 1 diabetes

Whereas in non-diabetic controls a high proportion of subjects had a low tortuosity score of 1 (45%), with a lower proportion of subjects in the tortuosity score 2 and 3 category (20%) (Figure 3A); in type 1 diabetes, the patients were more evenly distributed among the tortuosity scores 1-3 (Figure 3B). In type 1 diabetes, a relatively higher proportion of patients were stratified as having a tortuosity score 2 (27%) and 3 (37%) (Figure 3B). Accordingly, after correction for age and other cardiovascular risk factors in a backward multiple linear regression model, presence of type 1 diabetes (with non-diabetic controls as reference) was found to be associated with capillary tortuosity (Exp ß 0.6, 95% CI 0.1-1.0; $P = 0.02$).

Capillary tortuosity in type 2 diabetes

In contrast with non-diabetic controls, a minor proportion of patients with type 2 diabetes were stratified as having a low tortuosity score of 0 (1%) or 1 (13%) (Figure 3C). Whereas, a relatively large proportion of these patients had a high tortuosity score of 4 (27%). Indeed, after correction for age and other cardiovascular risk factors in a backward multiple linear regression model, the presence of type 2 diabetes (with non-diabetic controls as reference) was found to be a predictor of capillary tortuosity (Exp ß 0.5, 95% CI 0.2-0.8; $P = 0.001$).

CAD as assessed by MSCT in patients with diabetes

In the total sub-population of patients with diabetes, the mean CAC score was 213 ± 451. Overall, 39 (30%) patients with diabetes had an elevated CAC score of >100. Using MSCT angiography, presence of obstructive CAD (luminal narrowing ≥50%) was revealed in 31 (24%) patients with diabetes.

Relation of labial microvascular parameters with CAD in diabetes

The mean capillary density was higher in diabetic patients with a CAC score >100 (27 ± 4 per 0.63 mm^2) as compared to those with a CAC score in the range 0-100 (25 ± 4 per 0.63 mm^2) ($P = 0.04$) (Figure 4A). Similarly, after stratification according to MSCT angiography results, a higher mean capillary density was observed in diabetic patients with obstructive CAD (27 ± 4 per 0.63 mm^2), than in those without obstructive CAD (25 ± 4 per 0.63 mm^2) ($P = 0.02$) (Figure 4B).

As demonstrated in Figure 5A, none of the diabetic patients with a tortuosity score 0 (pinhead capillaries) had an elevated CAC score of >100. The prevalence of an elevated

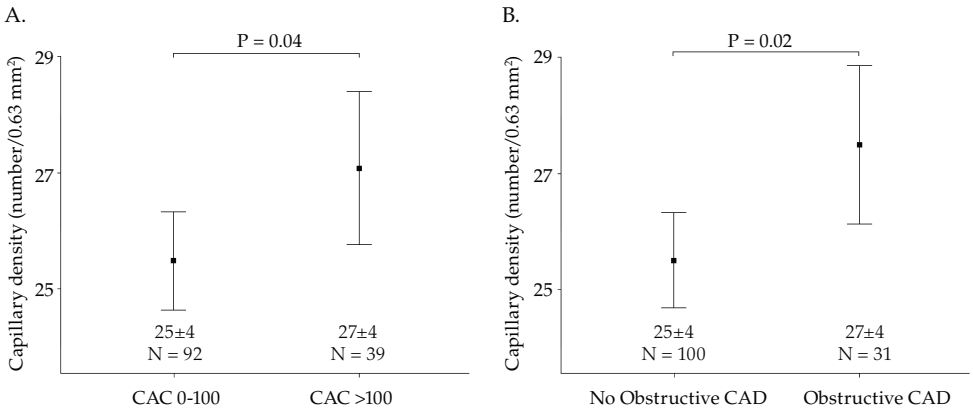

Figure 4. Relation of mean capillary density and CAD in asymptomatic diabetic patients. Mean capillary density was higher in patients with an increased CAC score of >100 (4A). Similarly, mean capillary density was higher in diabetic patients with obstructive CAD, as compared to those with no obstructive CAD (4B).

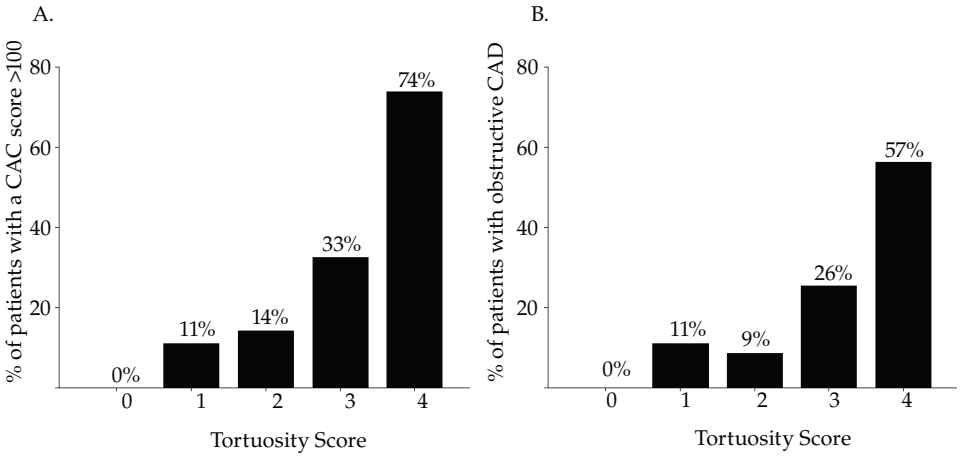

Figure 5. Relation of capillary tortuosity and CAD in asymptomatic diabetic patients. Relatively low prevalence of increased CAC scores of >100 were observed in patients with low tortuosity scores. On the contrary, the majority of patients with a high tortuosity score of 4 were revealed to have an increased CAC score of >100 (5A). A similar relation was observed between tortuosity score and the presence of obstructive CAD (5B).

Table 2. Predictors of a CAC score > 100, in patients with diabetes. Results of binary logistic regression analyses.

	Univariate Analysis		Multivariate Analysis (Backward)*	
	Exp ß (95% CI)	*P-value*	*Exp ß (95% CI)*	*P-value*
Age	1.12 (1.1-1.2)	<0.001	1.16 (1.1-1.3)	<0.001
Male sex	1.94 (0.9-4.2)	0.09	2.12 (0.6-7.7)	0.25
Diabetes duration (years)	1.02 (1.0-1.1)	0.16	-	-
Family history of CAD	1.14 (0.5-2.4)	0.73	-	-
Smoking	1.58 (0.7-3.6)	0.27	-	-
Body mass index (kg/m^2)	1.00 (0.9-1.1)	0.99	-	-
HbA1c	1.14 (0.9-1.4)	0.26	-	-
Micro-albuminuria	6.3 (2.4-15.4)	<0.001	9.12 (1.9-43.0)	0.01
Hypercholesterolemia	2.58 (1.1-6.3)	0.04	3.79 (1.0-14.8)	0.06
Hypertension	5.52 (2.2-13.8)	<0.001	-	-
Type 2 diabetes (vs. Type 1 diabetes)	1.29 (0.6-2.7)	0.50	0.12 (0.0-0.5)	0.01
Capillary density (number/0.63mm^2)	1.10 (1.0-1.2)	0.06	1.19 (1.0-1.4)	0.03
Capillary tortuosity (score 0-4)	2.65 (1.7-2.7)	<0.001	2.58 (1.3-5.2)	0.01

* All risk factors were entered in the model. Results are displayed for risk factors with a P-value ≤0.25. CAD = Coronary artery disease; HbA1c = glycated haemoglobin.

CAC score increased modestly to 11% and 14% with a tortuosity score of 1 and 2. The prevalence of an elevated CAC score increased further to 33% in those with a tortuosity score of 3. However, the most prominent increase in the prevalence of an elevated CAC score (74%) was observed in diabetic patients with highly tortuous capillaries (tortuosity score 4).

Likewise, a low tortuosity score of 0 excluded the presence of obstructive CAD in patients with diabetes (Figure 5B). A relatively low prevalence of obstructive CAD (11% and 9%) was observed in patients with a tortuosity score 1 and 2. In contrast, the prevalence of obstructive CAD more than doubled (26%) in patients with a tortuosity score 3. Importantly, a 57% majority of diabetic patients with a high tortuosity score of 4, were shown to have obstructive CAD.

Predictors of an elevated CAC score in diabetes

The results of binary logistic regression analysis for the evaluation of the risk factors associated with an elevated CAC score of >100 are provided in Table 2. Age, micro-albuminuria, hypercholesterolemia, hypertension and both capillary density and tortuosity were identified as potential predictors of an elevated CAC score, in patients with diabetes. Of note, after correction for other cardiovascular risk factors in a backward

Table 3. Predictors of obstructive CAD, in patients with diabetes. Results of binary logistic regression analyses.

	Univariate Analysis		Multivariate Analysis (Backward)*	
	Exp ß (95% CI)	*P-value*	*Exp ß (95% CI)*	*P-value*
Age	1.11 (1.1-1.2)	<0.001	1.11 (1.0-1.2)	0.001
Male sex	1.25 (0.6-2.8)	0.59	2.71 (0.8-9.5)	0.12
Diabetes duration (years)	1.00 (0.97-1.03)	0.94	-	-
Family history of CAD	1.46 (0.6-3.3)	0.36	-	-
Smoking	0.78 (0.3-2.0)	0.61	-	-
Body mass index (kg/m^2)	0.99 (0.9-1.1)	0.85	-	-
HbA1c	1.28 (1.0-1.7)	0.054	1.30 (0.9-1.9)	0.18
Micro-albuminuria	3.07 (1.2-7.9)	0.02	-	-
Hypercholesterolemia	1.57 (0.6-3.9)	0.33	-	-
Hypertension	2.65 (1.1-6.5)	0.03	-	-
Type 2 diabetes (vs. Type 1 diabetes)	2.23 (1.0-5.2)	0.06	-	-
Capillary density (number/0.63mm^2)	1.13 (1.0-1.2)	0.02	1.25 (1.1-1.5)	0.01
Capillary tortuosity (score 0-4)	2.20 (1.4-3.5)	0.001	1.91 (1.0-3.6)	0.04

* All risk factors were entered in the model. Results are displayed for risk factors with a P-value ≤0.25. CAD = Coronary artery disease; HbA1c = glycated haemoglobin.

multiple logistic regression model, capillary density (Exp ß 1.2, 95% CI 1.0-1.4; *P* = 0.03) as well as capillary tortuosity (Exp ß 2.6, 95% CI 1.3-5.2; *P* = 0.01), were shown to maintain a significant association with the presence of elevated CAC.

Using receiver operating characteristic (ROC) analysis a cut-off value of 24.9 per 0.63 mm^2 was identified for capillary density. This cut-off value yielded a negative- and positive predictive value of respectively 84% and 39% for predicting a CAC score >100. Of note, the positive predictive value improved from 39% to 66% in presence of a high tortuosity score of 4 besides a capillary density of ³24.9 per mm^2.

Predictors of obstructive CAD in diabetes

As illustrated in Table 3, age, micro-albuminuria, hypertension, capillary density and capillary tortuosity were found to be associated with obstructive CAD in patients with diabetes. Notably, analysis in a multivariate binary logistic model showed capillary density (Exp ß 1.3, 95% CI 1.1-1.5; *P* = 0.01) and capillary tortuosity (Exp ß 1.9, 95% CI 1.0-3.6; *P* = 0.04) to be independently associated with the presence of obstructive CAD (Table 3).

Using a cut-off value of 24.9 per 0.63 mm^2 for capillary density yielded a negative- and positive predictive value of respectively 89% and 32% for predicting obstructive CAD in

diabetes. The positive predictive value improved from 32% to 60% in presence of a high tortuosity score of 4 in addition to a capillary density of $^{3}24.9$ per mm^2.
Video clip examples of labial microcirculation as assesses by SDF are provided in the supplementary files (online availability via Diab Vasc Dis Res). Supplementary file 1 shows an example of the well ordered labial capillaries in the healthy. In comparison, the more tortuous and malformed capillaries often observed in diabetic patients with CAD are shown in supplementary files 2 and 3.

DISCUSSION

The main findings of the current study were as follows: firstly, the inter-observer (regression coefficients per observer 0.75 and 0.72) and intraobserver (regression coefficients per observer 0.80 and 0.72) for the assessment of capillary density using SDF imaging were reasonable. Similarly, a good inter-observer (agreement per observer 88%) and intraobserver (agreement per observer 90% and 88%) was found for the assessment of capillary tortuosity using SDF imaging. Secondly, after correction for age, gender and other cardiovascular risk factors, the presence of both type 1- and type 2 diabetes was found to be associated with an increased capillary density and tortuosity. Most importantly, in the sub-population of asymptomatic patients with diabetes, the mean labial capillary density was significantly higher in the presence of an increased CAC of >100 (P = 0.04) and in obstructive CAD (P = 0.02). Moreover, after correction for other cardiovascular risk factors, mean capillary density was shown to be an independent predictor of increased CAC (P = 0.03) and obstructive CAD (P = 0.01), in diabetes. Likewise, the prevalence of increased CAC and obstructive CAD increased with capillary tortuosity. Indeed, the capillary tortuosity score was also found to be an independent predictor of increased CAC (P = 0.01) and obstructive CAD (P = 0.04) on MSCT of asymptomatic patients with diabetes.

Assessment of microcirculation by SDF

Past studies of vital microcirculation were restricted to the use of contrast microscopy and laser Doppler. Non-invasive imaging of the superficial skin and mucous microcirculation was initially implemented using the orthogonal polarization spectral (OPS) device.[10] In OPS imaging the tissue embedding the microcirculation is illuminated with polarized green light. Illuminated light is absorbed by the haemoglobin in erythrocytes flowing through the tissue under investigation. As a result, the haemoglobin is used as the contrast agent, so that erythrocytes are imaged as dark globules in motion, against a white background. Consequently, the intravascular erythrocytes of perfused microvessels, rather than the microvessel walls are visualized. The imaging technique has been further modified in the SDF device to provide better visualization of the

microcirculation at capillary level.[15] In SDF, stroboscopic imaging partially prevents smearing of moving features such as the flowing red blood cells due to short illumination intervals. The microcirculatory image is more restricted from contamination by tissue surface reflection. Also, as compared to OPS, imaging by SDF has a shallower focusing depth. Therefore, the structures underlying the microcirculatory image field interfere to a lower extent.

The OPS and SDF imaging devices have been previously validated[10,16,17] and used to assess the functional anatomy of the sublingual, and nail fold microcirculation in critical care, and in patients with heart failure, sepsis and rheumatic diseases.[12,13] In the present study the assessment of the quantity (capillary density) and structure (tortuosity) of the labial capillaries using the SDF was validated. Evaluation of the labial microvascular network in non-diabetic controls and patients with diabetes, showed the capillary density and tortuosity to increase with the presence of diabetes. The increased labial capillary density and tortuosity may be a maker of microvascular disease.

Microvascular disease in diabetes

In diabetes, abnormal microvascular patterns have been described in nephropathy, retinopathy, and the myocardial capillaries.[18,19] Early morphological changes in the kidney of humans and animals with diabetic nephropathy include an increase in the number of glomerular capillaries as well as elongation and intermittent dilation and occlusion of the microvessels.[20-23] Alternatively, diabetic retinopathy can be classified as the early non-proliferative stage with microaneurysms and haemorrhages, or the later proliferative stage with formation of neovessels.[24] Furthermore, a study showed increased tortuosity of retinal vessels in presence of gestational diabetes.[25] Less information is available on the architecture of the myocardial microvessels in humans. However, in animal models, higher spatial capillary density and tortuosity have been observed in the myocardium in presence of diabetes.[26,27]

Various mechanisms have been proposed for the distortion of the microvascular network and the subsequent microvascular complications in patients with diabetes.[28,29] Hyperglycaemia is shown to promote exposure of endothelial cells to AGEs, resulting in protein kinase C activation, abnormal endothelial nitric oxide synthase expression and induction of Angiotensin-2 and vascular endothelial growth factor (VEGF).[30-32] Experimental studies suggest that VEGF may in turn stimulate the expression of adhesion molecules by endothelial cells and promote vascular inflammation, causing more adverse endothelial perturbations.[33,34] The overall molecular and functional changes result in the final sequelae of increased permeability of the microvessels and finally ischemia that drives unregulated angiogenesis.

Relation of microvascular disease and CAD

Micro- and macrovascular complications of diabetes share a number of pathogenic mechanisms.[8,30] Primarily, both processes include components of endothelial dysfunction and inflammation.[35-36] In addition, hypoxia induced angiogenesis is also increased in the vasa vasorum of the coronary arteries of patients with diabetes.[36] The corresponding neovasculature microangiopathy is suggested to accelerate atherosclerosis and predispose plaque rupture.[36] Thus, microvascular disease and CAD may be interconnected, with microvascular disease prompting atherosclerosis through hypoxia and changes in the vasa vasorum.

Accordingly, the majority of follow-up studies in patients with diabetes have found the presence of microvascular disease to increase risk of CAD irrespective of traditional cardiovascular risk factors.[5-7] During 5 years follow-up, Gall and colleagues found albuminuria to be a strong predictor cardiovascular mortality in patients with diabetes type 2 diabetes (HR 2.5 (1.1-5.8)).[5] Similarly, in the EURODIAB Prospective Cohort Study of 2,787 patients with type 1 diabetes, both albuminuria and peripheral neuropathy were shown to predict cardiovascular mortality, whereas retinopathy did not.[7] In contrast, in the Atherosclerosis Risk in Communities Study of patients with type 2 diabetes, the presence of diabetic retinopathy was found to be associated with a twofold risk of incident CAD and a threefold risk of fatal CAD, during an average follow-up of 7.8 years.[6] In line with these findings, we found a significant and independent relation between the labial parameters of microvascular disease, comprising of the capillary density and tortuosity, with increased CAC and obstructive CAD in patients with diabetes. In particular, a low capillary density of <24.9 per 0.63 mm^2 yielded a good negative predictive value for an increased CAC (84%) and obstructive CAD (89%). Also, labial capillary tortuosity score of 0 to 2, was associated with a low prevalence of an increased CAC (0 -14%) or obstructive CAD (0-11%). In contrast, in diabetic patients with a capillary tortuosity score of 4, a relatively high prevalence of increased CAC (74%) and obstructive CAD (57%) was observed.

Study limitations

A number of limitations must be acknowledged. First, the parameters of the microcirculation as assessed by SDF imaging only reflect characteristics of perfused capillaries. In the current study, influence of external factors on capillary recruitment was limited by standardizing the study environment. Also, flow alterations in the microcirculation due to external pressure were prevented by minimizing probe contact with the labial tissue during image acquisition. Herewith a good inter-session reproducibility of parameters of the microcirculation was observed in healthy non-diabetic controls. However, perfusion of capillaries may be less consistent in diabetes

as a consequence of functional and morphological changes. Secondly, the relation of labial capillary density and tortuosity with CAD could not be verified in the non-diabetic control group. As MSCT coronary angiography is accompanied with radiation exposure, it is not feasible to perform a similar assessment in asymptomatic subjects free of cardiovascular risk. Finally, the analysis was restricted to evaluation of the association between labial capillary density and tortuosity as assessed by SDF with traditional risk factors, as well as with the presence of CAD. However, the proatherogenic process which relates these microvascular parameters with CAD was not investigated.

Conclusion and future perspectives

The assessment of the labial capillary density and tortuosity, as markers of microvascular disease, is feasible and reproducible using the SDF imaging device. The labial capillary density and tortuosity increased with several traditional cardiovascular risk factors, micro-albuminuria and the presence of diabetes. A yet further increase in the labial capillary density and tortuosity was observed in diabetic patients with CAD. Assessment of the labial microvascular parameters using the non-invasive SDF handheld device may convey the potential to estimate the degree of vascular morbidity in patients with diabetes at bedside.

REFERENCES

1. American Diabetes Association: Diabetes statistics [article online], 2011. Available from http://www.diabetes.org/diabetes-statistics.jsp. Accessed 24 April 2011.
2. Raggi P, Bellasi A, Ratti C. Ischemia imaging and plaque imaging in diabetes: complementary tools to improve cardiovascular risk management. *Diabetes Care* 2005; 28: 2787-2794.
3. Young LH, Wackers FJ, Chyun DA, Davey JA, Barrett EJ, Taillefer R, et al. Cardiac outcomes after screening for asymptomatic coronary artery disease in patients with type 2 diabetes: the DIAD study: a randomized controlled trial. *JAMA* 2009; 301: 1547-1555.
4. Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. *N Engl J Med* 1984; 310: 356-60.
5. Gall MA, Borch-Johnsen K, Hougaard P, Nielsen FS, Parving HH. Albuminuria and poor glycemic control predict mortality in NIDDM. *Diabetes* 1995; 44: 1303-1309.
6. Cheung N, Wang JJ, Klein R, Couper DJ, Sharrett AR, Wong TY. Diabetic retinopathy and the risk of coronary heart disease: the Atherosclerosis Risk in Communities Study. *Diabetes Care* 2007; 30: 1742-1746.
7. Soedamah-Muthu SS, Chaturvedi N, Witte DR, Stevens LK, Porta M, Fuller JH; EURODIAB Prospective Complications Study Group. Relationship between risk factors and mortality in type 1 diabetic patients in Europe: the EURODIAB Prospective Complications Study (PCS). *Diabetes Care* 2008; 31: 1360-1366.
8. Orasanu G, Plutzky J. The pathologic continuum of diabetic vascular disease. *J Am Coll Cardiol* 2009; 53: S35-S42.
9. Moreno PR, Purushothaman KR, Sirol M, Levy AP, Fuster V. Neovascularization in human atherosclerosis. *Circulation* 2006; 113: 2245-2252.
10. Groner W, Winkelman JW, Harris AG, Ince C, Bouma GJ, Messmer K, et al. Orthogonal polarization spectral imaging: a new method for study of the microcirculation. *Nat Med* 1999; 5: 1209-1212.
11. De Backer D, Hollenberg S, Boerma C, Goedhart P, Büchele G, Ospina-Tascon G, et al. How to evaluate the microcirculation: report of a round table conference. *Crit Care* 2007; 11: R101.
12. Struijker-Boudier HA, Rosei AE, Bruneval P, Camici PG, Christ F, Henrion D, et al. Evaluation of the microcirculation in hypertension and cardiovascular disease. *Eur Heart J* 2007; 28: 2834-2840.
13. De Backer D, Creteur J, Dubois MJ, Sakr Y, Vincent JL. Microvascular alterations in patients with acute severe heart failure and cardiogenic shock. *Am Heart J* 2004; 147: 91-99.
14. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 1997; 20:1183.
15. Goedhart PT, Khalilzada M, Bezemer R, Merza J, Ince C.Sidestream Dark Field (SDF) imaging: a novel stroboscopic LED ring-based imaging modality for clinical assessment of the microcirculation. *Opt Express* 2007; 15:15101-15114.

16. Mathura KR, Vollebregt KC, Boer K, De Graaff JC, Ubbink DT, Ince C. Comparison of OPS imaging and conventional capillary microscopy to study the human microcirculation. *J Appl Physiol* 2001; 91: 74-78.
17. Boerma EC, Mathura KR, van der Voort PH, Spronk PE, Ince C. Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study. *Crit Care* 2005; 9: R601-R606.
18. Sasongko MB, Wang JJ, Donaghue KC, Cheung N, Benitez-Aguirre P, Jenkins A, et al. Alterations in retinal microvascular geometry in young type 1 diabetes. *Diabetes Care* 2010; 33: 1331-1336.
19. Nakagawa T, Kosugi T, Haneda M, Rivard CJ, Long DA. Abnormal angiogenesis in diabetic nephropathy. *Diabetes* 2009; 58: 1471-1478.
20. Osterby R, Nyberg G. New vessel formation in the renal corpuscles in advanced diabetic glomerulopathy. *J Diabet Complications* 1987; 1: 122-127.
21. Kanesaki Y, Suzuki D, Uehara G, Toyoda M, Katoh T, Sakai H, et al. Vascular endothelial growth factor gene expression is correlated with glomerular neovascularization in human diabetic nephropathy. *Am J Kidney Dis* 2005; 45: 288-294.
22. Osterby R, Bangstad HJ, Nyberg G, Rudberg S. On glomerular structural alterations in type-1 diabetes. Companions of early diabetic glomerulopathy. *Virchows Arch* 2001; 438: 129-135.
23. Wehner H, Nelischer G. Morphometric investigations on intrarenal vessels of streptozotocin-diabetic rats. *Virchows Arch A Pathol Anat Histopathol* 1991; 419: 231-235.
24. Mohamed Q, Gillies MC, Wong TY. Management of diabetic retinopathy: a systematic review. *JAMA* 2007; 298: 902-916.
25. Boone MI, Farber ME, Jovanovic-Peterson L, Peterson CM. Increased retinal vascular tortuosity in gestational diabetes mellitus. *Ophthalmology* 1989; 96: 251-254.
26. Factor SM, Minase T, Cho S, Fein F, Capasso JM, Sonnenblick EH. Coronary microvascular abnormalities in the hypertensive-diabetic rat. A primary cause of cardiomyopathy? *Am J Pathol* 1984; 116:9-20.
27. Saito F, Kawaguchi M, Izumida J, Asakura T, Maehara K, Maruyama Y. Alteration in haemodynamics and pathological changes in the cardiovascular system during the development of Type 2 diabetes mellitus in OLETF rats. *Diabetologia* 2003; 46: 1161-1169.
28. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001; 414: 813-820.
29. Stehouwer CD, Lambert J, Donker AJ, van Hinsbergh VW. Endothelial dysfunction and pathogenesis of diabetic angiopathy. *Cardiovasc Res* 1997; 34: 55-68.
30. Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation* 2006; 114: 597-605.

31. Stehouwer CD, Lambert J, Donker AJ, van Hinsbergh VW. Endothelial dysfunction and pathogenesis of diabetic angiopathy. *Cardiovasc Res* 1997; 34: 55-68.
32. Quagliaro L, Piconi L, Assaloni R, Da Ros R, Maier A, Zuodar G, et al. Intermittent high glucose enhances ICAM-1, VCAM-1 and E-selectin expression in human umbilical vein endothelial cells in culture: the distinct role of protein kinase C and mitochondrial superoxide production. *Atherosclerosis* 2005; 183: 259-267.
33. Miyamoto K, Khosrof S, Bursell SE, Moromizato Y, Aiello LP, Ogura Y, et al. Vascular endothelial growth factor (VEGF)-induced retinal vascular permeability is mediated by intercellular adhesion molecule-1 (ICAM-1). *Am J Pathol* 2000; 156: 1733-1739.
34. Aiello LP, Wong JS. Role of vascular endothelial growth factor in diabetic vascular complications. *Kidney Int Suppl* 2000; 77: S113-S119.
35. Zeiher AM, Drexler H, Wollschläger H, Just H. Endothelial dysfunction of the coronary microvasculature is associated with coronary blood flow regulation in patients with early atherosclerosis. *Circulation* 1991; 84: 1984-1992.
36. Fleiner M, Kummer M, Mirlacher M, Sauter G, Cathomas G, Krapf R, et al. Arterial neovascularization and inflammation in vulnerable patients: early and late signs of symptomatic atherosclerosis. *Circulation* 2004; 110: 2843-2850.

Summary and Conclusions

The general introduction (**Chapter 1**) of this thesis firstly outlines the concepts underlying atherosclerosis in diabetes. An overview of the dilemmas in risk stratification for coronary artery disease (CAD) in diabetes is provided. The clinical protocol that forms the basis for the studies presented in the thesis is described. Finally, the objective and outline of the thesis are explained.

PART 1

The first part of the thesis focuses on the nature of CAD in diabetes, and its presentation with different imaging modalities.

In Chapter 2, the relation of epicardial adipose tissue with CAD was assessed using CT angiography (CTA) in 190 patients. The study population comprised both non-diabetic and diabetic patients. The quantity of epicardial adipose tissue was an especially good predictor of any CAD. The relationship remained significant after correcting for diabetic status and other conventional CAD risk factors. An epicardial adipose tissue volume of 73 ml or more was shown to have a sensitivity and specificity of 77% and 70% for predicting a calcium score of >10, and 72% and 70% for presence of CAD on CTA. However, the quantity of epicardial adipose tissue did not significantly increase with the extent or severity of CAD.

The relation between the adipose tissue product adiponectin with parameters of CAD on CTA was explored in **Chapter 3.** Anomalous low and more variable adiponectin plasma levels are anticipated especially in patients with type 2 diabetes. The study was therefore conducted specifically in a type 2 diabetes subpopulation. Low plasma adiponectin was related with the presence of any atherosclerosis, obstructive atherosclerosis and atherosclerotic plaque burden. A plasma adiponectin of <4.5 mcg/ml resulted in a sensitivity and specificity of 80% and 71% for predicting obstructive atherosclerosis. Interestingly, analysis of plaque phenotype showed a predominant relation between low plasma adiponectin and the quantity of non-calcified plaques, which have been implicated in unstable CAD.

In **Chapter 4**, the nature of coronary atherosclerosis as assessed by CTA was compared in asymptomatic patients with type 1 and type 2 diabetes. No difference was observed in the average CAC score. However, the prevalence of obstructive atherosclerosis was higher in patients with type 2 diabetes. Also a higher mean number of atherosclerotic and obstructive plaques was observed in patients with type 2 diabetes. In addition, the percentage of noncalcified plaques was higher in patients with type 2 (66%) versus type 1 diabetes (27%). Consequently, in type 2 diabetes, a higher plaque burden was observed for each CAC score category.

Chapter 5 compared the diagnostic information obtained using CTA of the coronary arteries with functional data acquired using SPECT perfusion imaging, in 130 patients

with diabetes (type 1 and type 2). Thirty-five patients (27%) were shown to have obstructive epicardial CAD on CTA. Notably, in the remaining 95 patients free of obstructive epicardial CAD, abnormal myocardial perfusion was observed in yet 30 patients (32%). The endothelial function was assessed by the flow-mediated dilation of the brachial artery and compared in patients with normal and abnormal myocardial perfusion who were free of epicardial CAD. Flow-mediated dilatation was significantly lower in patients with abnormal myocardial perfusion (3.6% +/- 2.4%) as compared to those with normal myocardial perfusion (6.4% +/- 2.6%). This finding reflects abnormal myocardial perfusion as a consequence of reduced endothelial function in absence of obstructive CAD in a substantial proportion if asymptomatic patients with diabetes.

PART 2

In the second part of the thesis the value of non-invasive vascular measurements and cardiac imaging techniques was evaluated for risk stratification of CAD in diabetes.

Chapter 6 firstly reviews published data on the implementation of non-invasive vascular tools (carotid intima media thickness (CIMT), arterial stiffness and flow-mediated dilation) for risk stratification. Based on this overview it was concluded that CIMT and arterial stiffness as assessed by pulse wave velocity (PWV) show good reproducibility in diabetes, and are both increased in presence of clinically manifest CAD. However, limited data were available on the relation of these parameters with asymptomatic CAD in diabetic patients. Therefore, the value of CIMT and arterial stiffness for risk stratification of CAD in asymptomatic patients with diabetes remained to be examined. The use of flow-mediated dilation in clinical practice is limited due to poor reproducibility under variable external and physical circumstances. Secondly, the implementation of non-invasive cardiac imaging in patients with diabetes was reviewed. Previous studied showed CAC scores to be increased in presence of CAD. However, as compared to the general population, CAC scoring could possibly underestimate risk of future coronary events in diabetes. Anatomic imaging by CTA and functional testing by SPECT myocardial perfusion imaging had superior sensitivity and negative predictive value for presence of CAD in diabetes. However, risk stratification of all diabetic patients with CTA and/or SPECT myocardial perfusion imaging does not seem cost-effective and comprises radiation exposure. Therefore, we proposed an algorithm wherein the truly non-invasive and radiation free vascular measurements (CIMT and PWV) could serve as a primary risk stratification tool to select patients with type 2 diabetes who should undergo further assessment with CTA or SPECT.

Accordingly, **Chapters 7-9** focus on the value of CIMT and PWV as a primary risk stratification test in asymptomatic patients with diabetes.

In **Chapter 7**, assessment of CIMT in 150 asymptomatic patients with diabetes (type 1 and type 2) showed a significant relation with the presence and severity of CAD on CTA. The mean CIMT increased significantly from 0.58 +/- 0.08 mm in presence of normal coronary arteries to 0.67 +/- 0.12 mm in non-obstructive atherosclerosis and further to 0.75 +/- 0.12 mm in obstructive atherosclerosis. Receiver operating characteristics curve analysis yielded a sensitivity and specificity of 85% and 72%, with a CIMT cut-off value of 0.67 mm, for predicting obstructive CAD.

Chapter 8 evaluated the relation of CIMT with abnormal myocardial perfusion on SPECT, specifically in type 2 diabetes. Herein, increased CIMT emerged as an independent predictor of the presence and extent of abnormal myocardial perfusion. Importantly, only 3% of asymptomatic diabetic patients with normal CIMT values had severe myocardial perfusion abnormalities. Assessment of CIMT may therefore be useful to identify asymptomatic patients with diabetes at higher risk for CAD in need of further diagnostic testing using non-invasive cardiac imaging.

In **Chapter 9**, we evluated the value of the parameters of arterial stiffness as a primary risk stratification test in asymptomatic patients with diabetes. For this purpose the relation of the two non-invasive parameters of arterial stiffness, the PWV and the augmentation index, with the severity of myocardial perfusion abnormalities on SPECT was studied in 160 patients (type 1 and type 2). Both PWV and the augmentation index, were shown to increase with the severity of myocardial perfusion abnormalities on SPECT. However, after adjustment for age and other cardiovascular risk factors, PWV remained a significant predictor of severe myocardial perfusion abnormalities, whereas the augmentation index lost significance. This result suggests a potential for risk stratification for CAD through non-invasive assessment of arterial stiffness by PWV.

Subsequently, in **Chapter 10**, we compared the performance of the Framingham risk score, CIMT, PWV and CAC scoring as a primary risk stratification tool to identify the asymptomatic type 2 diabetes patients with functionally relevant CAD. Functionally relevant CAD defined as obstructive CAD on CTA and abnormal myocardial perfusion on SPECT was observed in 24% of patients. The Framingham risk score did not increase the AUC for predicting functionally relevant CAD (AUC 0.61, P=0.18). In contrast, the AUC increased significantly for PWV (AUC 0.68, P=0.03), CIMT (AUC 0.81, P<0.001) and CAC scoring (AUC 0.84, P<0.001). An excellent sensitivity and negative predictive value were observed with increased CIMT (83% and 95%) and a CAC score >100 (85% and 97%) for predicting functionally relevant CAD. As a consequence, CIMT and CAC scoring may be the preferred primary risk stratification tools for identification of asymptomatic patients with type 2 diabetes requiring further testing.

Chapter 11 describes a study exclusively in asymptomatic patients with type 1 diabetes. The presence and degree of myocardial perfusion abnormalities on SPECT were assessed.

Thereafter, we focused on the issue of risk stratification in this population. We observed abnormal myocardial perfusion in 41% of the patients, with 14% having severe defects. Significant predictors of extent of perfusion defects were age, micro-albuminuria and a CAC score >100. Severe perfusion defects were observed in yet 9% of patients without micro-albuminuria. On the other hand a CAC score <100 excluded severe perfusion defects. Additionally, CAC scoring was shown to have incremental value for predicting severe perfusion defects over age and the presence of micro-albuminuria (P=0.01). Consequently, abnormal myocardial perfusion is observed in a substantial number of asymptomatic patients with type 1 diabetes. CAC score assessment seems as the superior strategy to exclude the presence of silent severe CAD in type 1 diabetes.

PART 3

The third part of the thesis describes a novel technique, sidestream dark field imaging, for the assessment of the microcirculation at capillary level.

First, the technique was validated in **Chapter 12**. The reproducibility of labial capillary density and capillary tortuosity assessment was tested in 50 healthy volunteers. The interobserver correlation for the assessment of capillary density during the first session and second session were reasonable with a regression coefficient of 0.75 (P <0.001) and 0.72 (P <0.001) respectively. The intraobserver regression correlation coefficients for the assessment of capillary density were 0.80 (P <0.001) and 0.72 (P <0.001) for observer-1 and observer-2. The interobserver correlation for the assessment of capillary density during the first session and second session were reasonable with a regression coefficient of 0.75 (P <0.001) and 0.72 (P <0.001) respectively. The intraobserver regression correlation coefficients for the assessment of capillary density were 0.80 (P <0.001) and 0.72 (P <0.001) for observer-1 and observer-2. Therefore, we concluded that the assessment of the labial capillary density and tortuosity, as parameters of the microcirculation, is feasible and reproducible using the SDF imaging device.

In **Chapter 13**, we proceeded to study the parameters of labial microcirculation in patients with type 1- and type 2 diabetes as compared to healthy controls. Both type 1- and type 2 diabetes were associated with increased capillary density and tortuosity. The second part of the study was performed specifically in the diabetic population and evaluated the parameters of microcirculation in relation to CAD. In diabetic patients, the mean capillary density was an independent predictor of elevated CAC (P=0.03) and obstructive CAD on CTA (P=0.01). Using a cut-off mean capillary density of 24.9 (per 0.63 mm^2) the negative predictive value was 84% and 89% for elevated CAC and obstructive CAD. Likewise, capillary tortuosity was an independent predictor of increased CAC (P=0.01) and obstructive CAD (P=0.04). Therefore, it seems that the assessment of labial

microcirculation parameters using SDF conveys the potential to estimate vascular morbidity in patients with diabetes at bedside.

CONCLUSIONS

The primary objective of the thesis was to evaluate and compare various techniques and strategies for risk stratification of CAD in asymptomatic patients with diabetes.

The pathophysiologic pathway from obesity to CAD in diabetes is not fully understood. However, several markers of central adiposity show a relation with the degree of CAD. In this line, increased epicardial adipose tissue volume predisposes CAD. More specifically, the adipose tissue product adiponectin seems to be inversely related with the presence and degree of CAD in type 2 diabetes. A better understanding and employment of such markers could offer the possibility to distinguish the high risk diabetic patient at an early stage, prior to onset of vascular disease.

Dysfunction of the endothelium seems to occur in early stages of vascular disease. The consequent insufficient vasomotor response in the microvasculature may result in relative hypoperfusion. Therefore, in diabetic patients, myocardial perfusion defects prompted by endothelial dysfunction are sometimes observed in absence of obstructive CAD. In line with this observation, follow up studies have shown inducible myocardial perfusion defects to resolve in a majority of diabetic patients treated with antiatherogenic therapy. On the other hand, peristent hypoperfusion may result in morphological changes distinguished by proliferative neovascularization and distortion of the microvascular pattern. The SDF imaging device is validated for the non-invasive evaluation of these characteristics of the microvasculature. Using this novel technique, the assessment of capillary density and tortuosity, as markers of microvascular disease, is shown to be feasible and reproducible. Moreover, the presence of microvascular disease as assessed by SDF is associated with CAD in asymptomatic patients with diabetes.

In diabetic patients with atherosclerosis, CTA is able to define the extent, severity and composition of atherosclerotic lesions in the coronary arteries. Due to distinct pathophysiology and distinct patient profile, the nature of CAD as assessed by CTA differs in type 1- and type 2 diabetes. A relatively high proportion of calcified plaques is present in type 1 diabetes. Consequently, CAC score assessment seems the superior strategy to exclude the presence of severe CAD in asymptomatic patients with type 1 diabetes. Indeed, CAC scoring has incremental value over clinical risk factors for predicting severe CAD in type 1 diabetes. In type 2 diabetes, a higher plaque burden is observed for each CAC score category. Nevertheless, also in type 2 diabetes, a low CAC score performs well in excluding the presence of severe CAD accompanied with abnormal myocardial perfusion. However, risk stratification using the truly non-invasive

and radiation free CIMT performs equally well in predicting severe CAD accompanied with abnormal myocardial perfusion in type 2 diabetes.
A sequential approach comprising of CIMT or CAC scoring as a first step, followed by further diagnostic imaging if indicated, may possibly provide the most feasible and cost-effective approach for risk stratification of CAD in asymptomatic patients with diabetes. Thereby, a more patient tailored approach could be accomplished in patient management. However, prospective follow-up studies assessing the clinical outcome and cost-effectiveness of such algorithms are necessary to further define the role of non-invasive vascular tools and cardiac imaging techniques in management of patients with diabetes.

Samenvattingen en Conclusies

In de algemene inleiding (**Hoofdstuk 1**) van dit proefschrift wordt eerst een overzicht gegeven van onderliggende principes van atherosclerose vorming bij diabetes. Een overzicht van de dilemma's in risicostratificatie voor CAD bij diabetes wordt verstrekt. Het klinisch protocol dat de basis vormt voor de studies in dit proefschrift wordt beschreven. Tot slot worden de doelstelling en de opzet van het proefschrift toegelicht.

DEEL 1

Het eerste deel van het proefschrift richt zich op de aard van CAD bij diabetes, en de wijze waarop dit zich manifesteert bij verschillende beeldvormende modaliteiten. In **Hoofdstuk 2** werd de relatie tussen epicardiaal vetweefsel met CAD (beoordeeld met behulp van CTA) beschreven in 190 patiënten. De studiepopulatie bestond uit zowel niet-diabetische als diabetespatiënten. De hoeveelheid epicardiaal vetweefsel was een bijzonder goede voorspeller voor CAD. Het verband bleef significant na correctie voor diabetische status en andere conventionele CAD risicofactoren. Een epicardiaal vetweefsel volume van 73 ml of meer toonde een sensitiviteit en specificiteit van 77% en 70% voor het voorspellen van een calcium score van >10, en 72% en 70% voor de aanwezigheid van CAD op CTA. Echter, de hoeveelheid epicardiaal vetweefsel was niet significant gerelateerd aan de mate of ernst van CAD.

De relatie tussen het vetweefsel product adiponectine met parameters van CAD op CTA werd geëvalueerd in **Hoofdstuk 3**. Abnormaal lage en meer variabele adiponectine plasmaspiegels worden vooral bij patiënten met type 2 diabetes verwacht. De studie werd daarom specifiek in een type 2 diabetes subpopulatie uitgevoerd. Lage adiponectine spiegels waren gerelateerd aan de aanwezigheid van atherosclerose, obstructieve atherosclerose en hoeveelheid atherosclerotische plaque. Een adiponectine plasmaspiegel van <4,5 mcg/ml resulteerde in een sensitiviteit en specificiteit van 80% en 71% voor het voorspellen van obstructieve atherosclerose. Aanvullende analyse van plaque fenotype toonde een relatie tussen lage adiponectine spiegels met de aanwezigheid van niet-gecalcificeerde plaques welke vermoedelijk vaker bij instabiele CAD betrokken zijn.

In **Hoofdstuk 4** werd de uitgebreidheid, ernst en samenstelling van coronair atherosclerose zoals beoordeeld op CTA vergeleken tussen asymptomatische patiënten met type 1 en type 2 diabetes. Er werd geen verschil waargenomen in de gemiddelde calcium score. De prevalentie van obstructief atherosclerose was hoger bij patiënten met type 2 diabetes. Bij patiënten met type 2 diabetes werd ook een hoger gemiddeld aantal atherosclerotische plaques en obstructieve plaques waargenomen. Bovendien was het percentage niet-gecalcificeerde plaques hoger bij patiënten met type 2 (66%) diabetes in vergelijking met type 1 diabetes (27%). Derhalve werd bij type 2 diabetes een hogere plaque last waargenomen voor elke CAC score categorie.

In Hoofdstuk 5 werd in 130 patiënten met diabetes (type 1 en type 2) de diagnostische anatomische informatie verkregen middels CTA van de coronairen met de functionele data verkregen middels SPECT myocardperfusie. Vijfendertig patiënten (27%) bleken obstructief epicardiaal CAD te hebben op CTA. In de overige 95 patiënten zonder obstructief epicardiaal CAD, werd abnormale myocardperfusie waargenomen bij een opvallend hoog aantal patiënten (n=30, 32%). De endotheelfunctie werd beoordeeld middels FMD van de a. brachialis. Deze werd vergeleken in patiënten met normale- en abnormale myocardperfusie bij wie epicardiaal obstructief CAD uitgesloten was. FMD was significant lager bij patiënten met abnormale myocardperfusie (3,6% +/- 2,4%) in vergelijking met patiënten met normale myocardperfusie (6,4% +/- 2,6%). Deze bevindingen wijzen op frequent voorkomen van abnormale myocardperfusie als gevolg van verminderde endotheelfunctie bij asymptomatische patiënten met diabetes zonder obstructief epicardiaal CAD.

DEEL 2

In deel 2 van het proefschrift wordt de waarde van de niet-invasieve vasculaire metingen en beeldvormingstechnieken geëvalueerd voor risicostratificatie van CAD bij diabetes.

In **Hoofdstuk 6** werd een overzicht gegeven van artikelen die gepubliceerd zijn over de implementatie van niet-invasieve vasculaire metingen (CIMT, arteriële vaatstijfheid parameters en de FMD) voor risicostratificatie. Op basis van dit literatuuronderzoek werd geconcludeerd dat CIMT en arteriële vaatstijfheid zoals beoordeeld door PWV goede reproduceerbaarheid vertonen bij diabetes. De CIMT en PWV zijn beide verhoogd bij aanwezigheid van klinisch manifeste CAD bij diabetespatiënten. Er zijn echter beperkte gegevens beschikbaar over de relatie van deze parameters met asymptomatisch CAD bij diabetespatiënten. Daarom dient de waarde van CIMT en arteriële vaatstijfheid parameters voor CAD risicostratificatie bij asymptomatische diabetespatiënten nog te worden onderzocht. Het gebruik van FMD in de klinische praktijk bleek beperkt vanwege de slechte reproduceerbaarheid onder variërende externe en fysieke omstandigheden.

Tevens werd in dit hoofdstuk een overzicht gegeven over de implementatie van niet-invasieve cardiale beeldvormingstechnieken bij diabetespatiënten. Eerdere studies tonen in deze studiepopulaties verhoogde CAC scores in aanwezigheid van CAD. In vergelijking met de algemene bevolking onderschat de CAC score bij diabetes mogelijk het risico op toekomstige cardiale events als gevolg van CAD. Anatomische beeldvorming van de coronairen middels CTA en functionele beeldvorming middels SPECT myocard perfusiescintigrafie hebben een superieure sensitiviteit en negatief voorspellende waarde voor de aanwezigheid van CAD bij diabetes. Risicostratificatie bij alle diabetespatiënten met CTA en/of SPECT myocardperfusie lijkt echter niet kosteneffectief en stelt patiënten bloot aan ioniserende straling. Daarom hebben we een

algoritme voorgesteld waarin de werkelijk niet-invasieve en stralingsvrije vasculaire metingen (CIMT en PWV) als primaire sleutel dienen voor selectie van patiënten met type 2 diabetes die nader analyse dienen te ondergaan middels CTA of SPECT.
Om die reden wordt in de **Hoofdstukken 7-9** de waarde van CIMT en PWV als een primaire risicostratificatie test voor CAD beoordeeld bij asymptomatische patiënten met diabetes.
In **Hoofdstuk 7** werd bij 150 asymptomatische patiënten met diabetes (type 1 en type 2) een CIMT meting verricht. Deze toonde een significante relatie met de aanwezigheid en de ernst van CAD op CTA. De gemiddelde CIMT steeg substantieel van 0,58 +/- 0,08 mm bij patiënten met normale coronairen naar 0,67 +/- 0,12 mm in aanwezigheid van niet-obstructieve atherosclerose en verder naar 0,75 +/- 0,12 mm in aanwezigheid van obstructieve atherosclerose. ROC analyse leverde een sensitiviteit en specificiteit van 85% en 72% voor het voorspellen van obstructieve CAD bij een CIMT cut-off waarde van 0.67 mm.
In **Hoofdstuk 8** werd de relatie tussen CIMT met abnormale myocardperfusie op SPECT beoordeeld in een patiëntenpopulatie met enkel type 2 diabetes. Hierbij bleek een verhoogde CIMT een onafhankelijke voorspeller te zijn voor de aanwezigheid en uitgebreidheid van abnormale myocardperfusie. Van belang is dat ernstige myocardperfusie afwijkingen in slechts 3% van diabetespatiënten met een normale CIMT waard voorkwamen. Beoordelen van het CIMT kan daarom nuttig zijn om asymptomatische patiënten met diabetes te identificeren die een hoger risico op CAD lopen. Bij een verhoogd CIMT zou dan aanvullend diagnostisch onderzoek met behulp van niet-invasieve cardiale beeldvorming overwogen kunnen worden.
In **Hoofdstuk 9** werd de potentie van verschillende parameters van arteriële vaatstijfheid in de rol van een primaire risicostratificatietest in asymptomatische patiënten met diabetes onderzocht. Hiertoe werd de verhouding tussen de niet-invasief gemeten PWV en augmentatie index met de uitgebreidheid van myocardperfusie afwijkingen bij SPECT bestudeerd in 160 patiënten (type 1 en type 2). Zowel PWV als de augmentatie index bleken toe te nemen met de uitgebreidheid van myocardperfusie afwijkingen. Na correctie voor leeftijd en andere cardiovasculaire risicofactoren bleek alleen de PWV een significante voorspeller van ernstige myocardperfusie afwijkingen te zijn, terwijl de augmentatie index geen significante voorspellende waarde meer toonde. Dit resultaat suggereert een potentiële rol voor risicostratificatie voor CAD via een niet-invasieve bepaling van arteriële stijfheid middels PWV.
Vervolgens werden in **Hoofdstuk 10** de prestaties van de Framingham risicoscore, CIMT, PWV en CAC score als een primair instrument getoetst voor het herkennen van asymptomatische hoogrisico type 2 diabetes patiënten met functioneel relevant CAD. Functioneel relevant CAD werd gedefinieerd als aanwezigheid van zowel obstructief

CAD op CTA als abnormale myocardperfusie op SPECT. Dit werd waargenomen in 24% van de patiënten. De Framingham risicoscore toonde daarbij geen verhoging van de AUC voor het voorspellen van functioneel relevante CAD (AUC 0,61, p = 0,18). Daarentegen toonde de AUC wel significante stijging voor PWV (AUC 0,68, P = 0,03), CIMT (AUC 0,81, p <0,001) en CAC score (AUC 0,84, p <0,001). Een uitstekende sensitiviteit en negatief voorspellende waarde voor het voorspellen van functioneel relevante CAD werd waargenomen met een verhoogde CIMT (83% en 95%) en een CAC score >100 (85% en 97%). Daarom lijkt het verrichten van een CIMT meting of CAC score de geprefereerde primaire risicostratificatie methode voor identificatie van hoogrisico asymptomatische patiënten met type 2 diabetes.

Hoofdstuk 11 beschrijft een studie die uitsluitend bij asymptomatische patiënten met type 1 diabetes verricht is. De aanwezigheid en mate van myocardperfusie afwijkingen bij SPECT werd beoordeeld. Daarna hebben we ons gericht op de kwestie van de risicoanalyse bij deze populatie. Een abnormale myocardperfusie werd in 41% van de patiënten waargenomen, met in 14% van patiënten ernstige perfusiedefecten. Significante voorspellers van de mate van perfusiedefecten waren leeftijd, micro-albuminurie en een CAC score >100. In 9% van patiënten zonder micro-albuminurie werden alsnog ernstige perfusiedefecten waargenomen. Anderzijds kan bij een CAC score van <100 de aanwezigheid van ernstige perfusiedefecten volledig uitgesloten worden. Bovendien toonde de CAC score incrementele waarde boven de risicofactoren leeftijd en de aanwezigheid van micro-albuminurie in het voorspellen van ernstige perfusiedefecten (P = 0,01). Concluderend werd een abnormale myocardperfusie in een groot aantal asymptomatische patiënten met type 1 diabetes waargenomen. CAC score bepaling blijkt superieur te zijn in het uitsluiten van asymptomatische ernstige CAD bij type 1 diabetes.

DEEL 3

Het derde gedeelte van het proefschrift beschrijft een nieuwe techniek, SDF imaging, voor de beoordeling van de microcirculatie op capillair niveau.

Eerst werd de validatie van deze techniek beschreven in **Hoofdstuk 12**. De reproduceerbaarheid van de labiale capillairdichtheid en capillair tortuositeit middels SDF imaging werd getest in 50 gezonde vrijwilligers. De inter-correlatie voor de beoordeling van de capillairdichtheid tijdens de eerste sessie en de tweede sessie waren redelijk met een regressie coëfficiënt van respectievelijk 0,75 (P<0,001) en 0,72 (p<0,001). De intra-observer regressie correlatiecoëfficiënten voor de beoordeling van de capillairdichtheid waren 0,80 (p <0,001) en 0,72 (p <0,001) voor waarnemer-1 en waarnemer-2. De inter-correlatie voor de beoordeling van de capillairdichtheid tijdens de eerste sessie en de tweede sessie waren redelijk met een regressie coëfficiënt van respectievelijk 0,75

(P <0,001) en 0,72 (p <0,001). De intra-observer regressie correlatiecoëfficiënten voor de beoordeling van de capillairdichtheid waren 0,80 (p <0,001) en 0,72 (p <0,001) voor waarnemer-1 en waarnemer-2. Er werd geconcludeerd dat de beoordeling van de labiale capillairdichtheid en capillairtortuositeit met SDF, als parameters van de microcirculatie, haalbaar en reproduceerbaar lijkt.
In **Hoofdstuk 13** werden de parameters van de labiale microcirculatie bij patiënten met type 1 - en type 2 diabetes in vergelijking met gezonde controles bestudeerd. Zowel type 1 - als type 2 diabetes bleken geassocieerd te zijn met verhoogde capillairdichtheid en capillairtortuositeit. Het tweede deel van de studie werd uitgevoerd bij in de subpopulatie van patiënten met diabetes. De relatie tussen CAD met de parameters van de microcirculatie bij diabetespatiënten werd onderzocht. Bij diabetespatiënten bleek de gemiddelde capillairdichtheid een onafhankelijke voorspeller van zowel verhoogde CAC score (P=0,03) als obstructief CAD op CTA (P = 0.01). Op basis van een capillairdichtheid cut-off waarde van 24,9 (per 0.63 mm2) werden negatief voorspellende waarden van respectievelijk 84% en 89% berekend voor een verhoogde CAC score en voor obstructief CAD. Ook bleek de capillairtortuositeit een onafhankelijke voorspeller voor verhoogde CAC (P = 0.01) en obstructief CAD (P = 0.04). Deze waarnemingen suggereren dat analyse van de labiale microcirculatie parameters met behulp van SDF een potentieel klinisch toepasbaar instrument vormt voor het in schatten van vasculaire morbiditeit bij patiënten met diabetes.

CONCLUSIES

De primaire doelstelling van het proefschrift was om verschillende technieken en strategieën voor risicostratificatie van CAD bij asymptomatische patiënten met diabetes te evalueren en te vergelijken.
De pathofysiologische weg van overgewicht naar CAD bij diabetespatiënten is niet volledig begrepen. De verschillende markers van centrale adipositas lijken echter een relatie te tonen met de uitgebreidheid van CAD. Een verhoogde epicardiaal vetweefsel volume toont dan ook een predispositie voor CAD. Daarnaast toont het vetweefsel product adiponectine juist een omgekeerd evenredig relatie met de aanwezigheid en de mate van CAD in type 2 diabetes. Een beter begrip en toepassing van dergelijke markers zou het herkennen van een hoogrisico diabetespatiënt in een vroeg stadium en vóór aanvang van vaatziekte mogelijk kunnen maken.
Endotheeldysfunctie lijkt al in een vroeg stadium van vaatziekte plaats te vinden. De daaruit voortvloeiende ontoereikende vasomotorische respons in de microvasculatuur kan resulteren in relatieve hypoperfusie. Bij diabetespatiënten lijken derhalve in afwezigheid van obstructief CAD myocard perfusiedefecten waargenomen te worden als gevolg van endotheeldysfunctie. Eerdere follow-up studies bij diabetespatiënten

hebben dan ook herstel van myocard perfusie defecten aangetoond na behandeling met medicamenteuze anti-atherogene therapie. Anderzijds kan persisterende hypoperfusie ook tot morfologische veranderingen leiden die gekenmerkt worden door proliferatieve neovascularisatie en vervorming van de microvasculaire patroon. De SDF beeldvorming is gevalideerd voor de niet-invasieve evaluatie van deze eigenschappen van de microvasculatuur. Toepassing van deze nieuwe techniek blijkt haalbaar en reproduceerbaar voor de beoordeling van capillaire dichtheid en tortuositeit als kenmerken van microvasculaire aantasting. Bovendien wordt de aanwezigheid van microvasculaire kenmerken zoals beoordeeld door SDF geassocieerd met CAD bij asymptomatische diabetes patiënten.

Bij diabetische patiënten met atherosclerose kan CTA de uitgebreidheid, de ernst en samenstelling van atherosclerotische laesies in de coronairen definiëren. Wegens verschillen in pathofysiologie en risicoprofiel, verschilt ook de aard van CAD zoals beoordeeld door CTA in type 1 - en type 2 diabetes. Een relatief groot aandeel van CAD bestaat uit gecalcificeerde plaques bij type 1 diabetes. Hierdoor lijkt evaluatie middels CAC score de superieure strategie om de aanwezigheid van ernstige CAD bij asymptomatische patiënten met type 1 diabetes uit te sluiten. Een CAC score verrichting heeft dan ook incrementele waarde over klinische risicofactoren voor het voorspellen van ernstige CAD bij type 1 diabetes. Bij type 2 diabetes is een hogere plaque last waargenomen voor elke CAC score categorie. Een lage CAC score sluit van de aanwezigheid van ernstig CAD gepaard met abnormale myocardperfusie ook bij type 2 diabetes uit. Evenwel presteert risicostratificatie met behulp van de niet-invasieve en stralingsvrije CIMT even goed in het voorspellen van ernstige CAD gepaard met abnormale myocardperfusie bij type 2 diabetes.

Een sequentiële aanpak bestaande uit CIMT of CAC score als een eerste stap, gevolgd door verdere diagnostische beeldvorming op indicatie, kan zorgen voor de meest haalbare en kosteneffectieve aanpak voor risicostratificatie van CAD bij asymptomatische patiënten met diabetes. Daardoor zou een meer patiënt gerichte benadering kunnen worden bereikt in het beheer van patiëntenzorg. Prospectieve follow-up trials voor het beoordelen van de klinische uitkomst en de kosteneffectiviteit van dergelijke algoritmen zijn noodzakelijk om de rol van niet-invasieve vasculaire instrumenten en cardiale beeldvormingstechnieken in het kader van behandeling van patiënten met diabetes verder te definiëren.

Publications
Acknowledgements
Curriculum Vitae

Publications

Non-invasive cardiac imaging techniques and vascular tools for the assessment of cardiovascular disease in type 2 diabetes mellitus.
Djaberi R, Beishuizen ED, Pereira AM, Rabelink TJ, Smit JW, Tamsma JT, Huisman MV, Jukema JW.
Diabetologia. 2008; 51:1581-1593

Relation of epicardial adipose tissue to coronary atherosclerosis.
Djaberi R, Schuijf JD, van Werkhoven JM, Nucifora G, Jukema JW, Bax JJ.
Am J Cardiol. 2008; 102:1602-1607

Endothelial dysfunction in diabetic patients with abnormal myocardial perfusion in the absence of epicardial obstructive coronary artery disease.
Djaberi R, Roodt Jo, Schuijf JD, Rabelink TJ, de Koning EJ, Pereira AM, Stokkel MP, Smit JW, Bax JJ, Jukema JW.
J Nucl Med. 2009; 50:1980-1986

Usefulness of carotid intima-media thickness in patients with diabetes mellitus as a predictor of coronary artery disease.
Djaberi R, Schuijf JD, de Koning EJ, Rabelink TJ, Smit JW, Kroft LJ, Pereira AM, Scholte AJ, Spaans M, Romijn JA, de Roos A, van der Wall EE, Jukema JW, Bax JJ.
Am J Cardiol. 2009; 104:1041-1046

Relation between Framingham risk categories and the presence of functionally relevant coronary lesions as determined on multislice computed tomography and stress testing.
Nucifora G, Schuijf JD, van Werkhoven JM, **Djaberi** R, van der Wall EE, de Roos A, Scholte AJ, Schalij MJ, Jukema JW, Bax JJ.
Am J Cardiol. 2009; 104:758-763

Differences in atherosclerotic plaque burden and morphology between type 1 and 2 diabetes as assessed by multislice computed tomography.
Djaberi R, Schuijf JD, Boersma E, Kroft LJ, Pereira AM, Romijn JA, Scholte AJ, Jukema JW, Bax JJ.
Diabetes Care. 2009; 32:1507-1512

Prevalence of coronary artery disease across the Framingham risk categories: coronary artery calcium scoring and MSCT coronary angiography.
Nucifora G, Schuijf JD, van Werkhoven JM, Jukema JW, **Djaberi** R, Scholte AJ, de Roos A, Schalij MJ, van der Wall EE, Bax JJ.
J Nucl Cardiol. 2009; 16:368-375

Non-invasive imaging: Non-invasive assessment of coronary artery disease in diabetes.
Van de Veire NR, **Djaberi** R, Schuijf JD, Bax JJ.
Heart. 2010; 96:560-572

Increased carotid intima-media thickness as a predictor of the presence and extent of abnormal myocardial perfusion in type 2 diabetes.
Djaberi R, Schuijf JD, Jukema JW, Rabelink TJ, Stokkel MP, Smit JW, de Koning EJ, Bax JJ.
Diabetes Care. 2010; 33:372-374

Usefulness of hypertriglyceridemic waist phenotype in type 2 diabetes mellitus to predict the presence of coronary artery disease as assessed by computed tomographic coronary angiography.
de Graaf FR, Schuijf JD, Scholte AJ, **Djaberi** R, van Velzen JE, Roos CJ, Kroft LJ, de Roos A, van der Wall EE, Wouter Jukema J, Després JP, Bax JJ.
Am J Cardiol. 2010; 106:1747-1753

Relationship between vascular stiffness and stress myocardial perfusion imaging in asymptomatic patients with diabetes.
Roos CJ, **Djaberi** R, Schuijf JD, de Koning EJ, Rabelink TJ, Smit JW, Pereira AM, Al Younis I, van der Hiel B, Scholte AJ, Bax JJ, Jukema JW.
Eur J Nucl Med Mol Imaging. 2011; 38:2050-2057

Multimodality imaging in diabetic heart disease.
Ng AC, Delgado V, **Djaberi** R, Schuijf JD, Boogers MJ, Auger D, Bertini M, de Roos A, van der Meer RW, Lamb HJ, Bax JJ.
Curr Probl Cardiol. 2011; 36:9-47

Subclinical left ventricular dysfunction and coronary atherosclerosis in asymptomatic patients with type 2 diabetes.
Scholte AJ, Nucifora G, Delgado V, **Djaberi** R, Boogers MJ, Schuijf JD, Kharagjitsingh AV, Jukema JW, van der Wall EE, Kroft LJ, de Roos A, Bax JJ.
Eur J Echocardiogr. 2011; 12:148-155

Relationship between left ventricular diastolic function and arterial stiffness in asymptomatic patients with diabetes mellitus.
Roos CJ, Auger D, **Djaberi** R, de Koning EJ, Rabelink TJ, Pereira AM, Bax JJ, Delgado V, Jukema JW, Scholte AJ.
Int J Cardiovasc Imaging. 2013; 29:609-616

Non-invasive assessment of microcirculation by sidestream dark field imaging as a marker of coronary artery disease in diabetes.
Djaberi R, Schuijf JD, de Koning EJ, Wijewickrama DC, Pereira AM, Smit JW, Kroft LJ, Roos Ad, Bax JJ, Rabelink TJ, Jukema JW.
Diab Vasc Dis Res. 2013; 10:123-134

Very-low-calorie diet increases myocardial triglyceride content and decreases diastolic left ventricular function in type 2 diabetes with cardiac complications.
Jonker JT, **Djaberi** R, van Schinkel LD, Hammer S, Bus MT, Kerpershoek G, Kharagjitsingh AV, Romijn JA, Bax JJ, Jukema JW, de Roos A, Smit JW, Lamb HJ.
Diabetes Care. 2014; 37:e1-2

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Curriculum Vitae

The author of this thesis was born on June 14, 1979 in Tehran, Iran. After graduating from the International School of The Hague in 1997, she studied Medicine at Leiden University. During her study she was involved in a research project investigating the aortic root dilatation in children who had undergone a Ross procedure at the Center for Congenital Heart Abnormalities Amsterdam-Leiden (CAHAL) (supervisor: prof. dr. J. Ottenkamp).

After receiving her medical degree in 2004, she worked at the Department of Cardiology in the HAGA ziekenhuis, The Hague, for 12 months (educational heads: dr. G.A. van der Kley and dr. B.J.M. Delemarre). In 2006 she started a research fellowship on cardiovascular disease in diabetes at the Department of Cardiology of the Leiden University Medical Center (supervisors: prof. dr. J.W. Jukema and prof. dr. J.J. Bax). The results of studies performed during this research period are described in the present thesis.

In July 2009, she started her training in Internal Medicine at the HAGA ziekenhuis, The Hague (educational head: dr. M.O. van Aken). Her traineeship was continued at the Department of Cardiology at Groene Hart ziekenhuis, Gouda (educational head: dr. M.W.J. van Hessen) and the Department of Cardiology at Leiden University Medical Center (educational head: prof. M.J. Schalij).