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.