Chapter 1
General introduction and aims of the thesis

Stefanie A. de Boer
GENERAL INTRODUCTION

Diabetes a health-care burden worldwide

Diabetes has become a worldwide public health problem that affects more than 400 million people. Overall, the global prevalence of diabetes in the adult population has nearly doubled, from 4.7% in 1980 to 8.5% in 2014, and this rate continues to increase.\(^1\) This is a result of increasing obesity and physical inactivity, aging, population growth, and urbanization. As the prevalence of diabetes is increasing worldwide, it is no longer a disease of the wealthy. Consequently, the United Nations have decided that diabetes is one of the four priority non-communicable diseases (NCDs) to be targeted for action.\(^2\) Diabetes and the other priority NCDs (cancer, chronic lung diseases and cardiovascular disease (CVD)) are responsible for almost 70% of all deaths worldwide. The goal is to decrease the mortality rates of these four priority NCDs by 25% by 2025.

In the Netherlands the number of people currently diagnosed with diabetes is estimated to be 1.1 million.\(^3\) Taking into account the number of people living with undiagnosed diabetes, the number of people living with diabetes is 1.2 million. This represents 1 in 14 people having diabetes in the Netherlands. Moreover, it was recently calculated that, in the Netherlands, for a non-diabetic individual aged 45 years and older the lifetime risk of developing diabetes is one in three.\(^4\)

Diabetes clinical presentation and risk factors

Diabetes mellitus is a chronic metabolic disease marked by high levels of glucose in the blood (hyperglycemia). Hyperglycemia occurs when the pancreas does not secrete enough insulin, or when the body is resistant to insulin activity. Insulin is the major regulator of glucose metabolism, by stimulating the uptake of glucose by muscles and organs and maintaining glucose production from the liver. When a patient becomes insulin resistant, the insulin binding to its receptor on the surface of a cell is normal but the insulin signaling within the cell is abnormal.\(^5\) Insulin resistance is often seen in cells in the liver, skeletal muscle, adipose tissue and endothelium. To overcome insulin resistance, the secretion of insulin is increased; this is known as compensatory hyperinsulinemia. Diabetes develops when the compensatory hyperinsulinemia is unable to maintain normal glucose levels.\(^6\) The chronic hyperglycemic state leads, over time, to late complications including tissue damage, organ dysfunction and ultimately organ failure. The eyes, kidneys, nerves, heart and blood vessels are particularly affected by the hyperglycemic state. As a result of the organ complications the risk of premature mortality increases.\(^1,6\)

Diabetes is usually classified into two types, based on etiologic differences.\(^6\) Type 1 diabetes (5-10% of those with diabetes) is the result of an autoimmune destruction of the β-cells of the pancreas, leading to absolute insulin deficiency. Clinical presentation often includes symptoms of polyuria, polydipsia, and unexplained weight loss.\(^6\)
Type 2 diabetes (90-95% of those with diabetes) is the result of a relative lack of insulin (β-cell dysfunction), insulin resistance, or both. The risk factors for the development of type 2 diabetes are multifactorial. Ethnicity, family history of diabetes, increasing age, overweight and obesity, abdominal obesity, diet and physical inactivity are all contributing factors. For example, the risk of type 2 diabetes occurs at a lower BMI in Asian populations than in Europeans. The clinical presentation of type 2 diabetes may be similar to that of type 1 diabetes, but is often asymptomatic. Because hyperglycemia develops progressively, the early stage is often not severe enough to lead to overt symptoms. Consequently, type 2 diabetes is often undiagnosed for several years.

According to the American Diabetes Association, the criteria for the diagnosis of diabetes are fasting plasma glucose ≥7.0 mmol/l (≥126 mg/dL), and/or a random plasma glucose ≥11.1 mmol/l (200 mg/dL), and/or a glycated haemoglobin (HbA1c) ≥6.5% (≥48 mmol/l). A fasting plasma glucose of 5.6 to 7.0 mmol/l (100 to 125 mg/dL), and/or a random plasma glucose of 7.8 to 11.0 mmol/l (140 to 199 mg/dL), and/or a HbA1c of 5.7% (≥48mmol/l ) to 6.4% (≥48mmol/l ) are defined as prediabetes. People with prediabetes should be tested yearly for diabetes.

Cardiovascular risk factors in diabetes
Compared with non-diabetic people, people with type 2 diabetes are disproportionately affected by CVD, such as myocardial infarction and stroke. Hence, the presence of diabetes is a major risk factor for CVD and premature mortality. Moreover, people with prediabetes are already at an increased risk of CVD. However, the pathogenesis of CVD in type 2 diabetes is complex.

One risk factor, often mentioned as a possible explanation for the increased cardiovascular (CV) risk in diabetes, is the chronic hyperglycemic state. As hyperglycemia is mostly asymptomatic, it can remain undiagnosed and consequently untreated for several years. During hyperglycemia, proteins or lipids become glycated after exposure to sugars, and advanced glycation end products (AGEs) are formed. Also monocyte adhesion to arterial endothelial cells is enhanced. Both processes promote the development of atherosclerosis and ultimately CVD. However, in type 2 diabetes, compared with type 1 diabetes, hyperglycemia itself is not a very strong risk factor for CVD. For instance, an increment of 1 unit (%) of HbA1c increased CV mortality by 52.5% in people with type 1 diabetes, and only by 7.5% in people with type 2 diabetes, although the overall mortality rate was similar. Furthermore, CV benefits of intensive glucose-lowering therapy in type 2 diabetes have not been unequivocally demonstrated in clinical trials. In addition, it has been suggested that not hyperglycemia but the related insulin resistance is a more important risk factor for CVD. Insulin resistance and the accompanying hyperinsulinemia are both linked to an increased CV risk. In addition, insulin resistance is associated with prothrombotic, proinflammatory and atherogenic abnormalities such as impaired endothelial function,
subclinical inflammation, changes in adipokines, dyslipidemia, increased levels of free fatty acids, and changes in mediators of thrombosis and fibrinolysis. For example, people with insulin resistance already display endothelial dysfunction and inelastic arteries, indicating vascular dysfunction. Type 2 diabetes further enhances this vascular dysfunction, resulting in a more stiffened artery and a higher risk of CVD.

Furthermore, insulin resistance increases due to other CV risk factors, including obesity, abdominal obesity, elevated blood pressure, elevated total triglycerides, and low HDL cholesterol. For example, abdominal obesity itself causes some degree of insulin resistance by changing the secretion of adipokines (cytokines secreted by adipose tissue) like leptin and adiponectin. Moreover, insulin resistance is triggered by an excess accumulation of intracellular triglycerides. Hence, it is not clear whether insulin resistance is a causal factor or simply a marker for the increased CV risk.

The well-known classic risk factors for CVD, such as hypertension, dyslipidemia and cigarette smoking, are significant risk factors of CV mortality in people, both with or without diabetes. However, the absolute risk of CV mortality related to every kind or risk factor is at least twice as great in people with diabetes as in people without diabetes. Therefore, the increased CV risk in diabetes cannot be attributed mainly to these classic risk factors. Other well-known CVD risk factors include obesity, abdominal obesity and physical inactivity, although not every obese person is at high risk of CVD and diabetes. However, abdominal obesity, in particular an excess of intra-abdominal visceral adipose tissue, is undoubtedly associated with CVD risk. Nowadays, visceral adipose tissue is recognized not only as a storage of lipids but also as an endocrine organ with adipocytes secreting bioactive factors and pro-inflammatory cytokines commonly known as adipokines. Adipokines promote endothelial dysfunction, insulin resistance, hypercoagulability, and ultimately atherosclerosis.

Overall, type 2 diabetes is considered to be a low-grade chronic inflammatory disease. Moreover, insulin resistance, which is common in type 2 diabetes, is also linked to chronic inflammation. Atherosclerosis is, like diabetes and insulin resistance, recognized as a chronic inflammatory disease. Therefore, inflammation may be an antecedent of both type 2 diabetes and premature atherosclerosis.

**DPP-4 a new link between type 2 diabetes and cardiovascular risk?**

Although inflammation may precede both type 2 diabetes and premature atherosclerosis, the exact interplay between the immune system, the pathophysiology of type 2 diabetes, and CVD is not yet fully understood. Recent data suggest a pathophysiological link between the enzyme dipeptidyl peptidase 4 (DPP-4), endothelial dysfunction and low-grade chronic inflammation, all of which are directly linked to the pathogenesis and clinical manifestations of type 2 diabetes and atherosclerosis.
DPP-4 (also known as CD26) is an enzyme which can be in soluble form in plasma or incorporated into the plasma membrane of many cell types, including endothelial cells. In addition, the main endogenous source of DPP-4 is probably the endothelial cell. The release of DPP-4 from the membrane can be enhanced by stimuli like insulin resistance and chronic low-grade inflammation. DPP-4 activity is increased during hyperglycemia. In people with drug-naïve type 2 diabetes, compared with healthy controls, DPP-4 activity is significantly increased, and it decreases after active glucose control. Furthermore, it was found that DPP-4 activity independently predicted the risk of developing prediabetes and type 2 diabetes in normoglycemic people after 4 years of follow-up. DPP-4 rapidly degrades incretins such as glucagon-like peptide 1 (GLP-1), which controls glucose dependent insulin secretion. GLP-1 has several additional extra-glycemic effects, which have been associated with favorable CV effects. However, DPP-4 has several additional effects beyond GLP-1 degradation.

DPP-4 is widely distributed in tissues such as the kidney, intestines, adipose tissue, endothelial cells and bone marrow derived cells. DPP-4 has been shown to cleave multiple substrates, many of which influence the CV system. One example substrate is the chemokine stromal cell-derived factor-1α (SDF-1α), which is responsible for the recruitment of endothelial progenitor cells (EPCs). EPCs are known to play a role in vascular repair. Moreover, DPP-4 is expressed on blood T cells, when activated cytokines like IL-2, IL-10, IL-12 and IFN-γ are released. Therefore, DPP-4 appears to regulate several physiological pathways, and not only affecting insulin secretion but also inflammation, immunity, and vascular function. Considering the higher DPP-4 activity level in people with diabetes as compared to non-diabetics, it can therefore be assumed that DPP-4 constitutes a new link between type 2 diabetes and CV risk.

In order to reduce glycemic levels by prolonging the half-life of incretins, DPP-4 inhibitors (commonly referred to as gliptins) have been developed for the treatment of type 2 diabetes. Moreover, DPP-4 inhibition has several other effects on physiological pathways, which may reduce the CV risk, as shown in Figure 1.

Treatment of diabetes
The treatment modalities for type 2 diabetes are numerous. Not all risk factors for type 2 diabetes are modifiable, such as ethnicity and age; however, others like obesity are. Therefore, the keys to treatment and prevention of type 2 diabetes are diet and lifestyle changes. For instance, insulin resistance may improve with weight reduction and increased physical activity. However, if hyperglycemia is not satisfactorily controlled by diet and lifestyle changes, drugs may be needed. To monitor the treatment of diabetes the measurement of HbA1c is the method of choice. HbA1c reflects average glycemia over several months and is related to the risk of diabetes complications. The glycemic target for adults with diabetes is a HbA1c of <7.0% (53 mmol/mol).
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Figure 1 | Mechanisms underlying the beneficial effects of DPP4 inhibitors on the cardiovascular system.

Although this target can be individualized based on the effect and safety of the treatment, in which case the target may be more or less stringent. For example, severe hypoglycemia, which can be a complication of aggressive glycemic control, is a serious side effect that should be prevented, particularly in the frail elderly. Therefore, setting higher HbA1c control targets is sometimes necessary. This is in contrast to cases of newly diagnosed young adults without important comorbidities, for whom more stringent HbA1c control targets are determined as optimal. Moreover, the United Kingdom Prospective Diabetes Study (UKPDS), that included about 5000 recently diagnosed people with type 2 diabetes, compared intensive glycemic treatment with conventional treatment. The UKPDS showed a long-lasting CV benefit of intensive glycemic control, even after intensive glycemic control had relented. This benefit, long after treatment given in an early phase of a disease, is described as the "legacy effect". Therefore, achieving glycemic control as soon as possible, particularly early in the disease, may account for long-term beneficial CV effects.

Currently there are several types of drugs that work in different ways to lower blood glucose levels. Ideally, since people with type 2 diabetes suffer from an increased risk of CVD, drugs to treat diabetes should, beyond their glycemic effects, also offer CV protection. Metformin is recommended in the guidelines as the first line drug of choice because it has beneficial effects on insulin resistance. Furthermore, metformin monotherapy has a low risk on hypoglycemia, neutral effect on weight, and low costs. If the HbA1c target is not achieved after the start of metformin, the start of another antihyperglycemic drug along with metformin is recommended. Other treatment options commonly used with metformin are: a sulfonylurea, thiazolidinedione, DPP-4 inhibitor, SGLT2 inhibitor, GLP-1 receptor agonist and insulin. Compared with the effects of other active-comparator drugs, metformin's beneficial effects on CV events are not unconditional. Although metformin is associated with a reduction of CV events when compared with placebo or no treatment, the residual CV risk is still high. Therefore, other anti-diabetic drugs than metformin should be investigated for their greater ability to reduce CV risk. For instance, DPP-4 inhibitors, which are a relatively new class of oral anti-diabetic drugs, may have favorable CV effects, as shown in Figure 2. Experimental studies have shown that DPP-4 inhibitors reduce atherosclerotic plaque area and macrophage accumulation. This is in line with the suggestion that DPP-4 forms a link between diabetes and CVD. Furthermore, DPP-4 inhibitors are effective in reducing HbA1c without inducing hypoglycemia. The CV safety (non-inferiority) of DPP-4 inhibitors (i.e. alogliptin, saxagliptin, sitagliptin) has been demonstrated in three published CV outcome trials (EXAMINE, SAVOR, TECOS), although these trials were unable to demonstrate a clear CV benefit. However, these trials were designed to demonstrate CV safety, and DPP-4 inhibitors were added to usual care in people with established CVD. Nevertheless, randomized controlled trials that investigate the effect of DPP-4 inhibitors on favorable CV effects, like reducing atherosclerotic plaque areas, are lacking.
Finally, it may be clear that the increased CV risk in diabetes is the result of the interaction of various risk factors, of which hyperglycemia is only one. Therefore, glycemic control is not the only treatment target for type 2 diabetes. Treatment of other CV risk factors like hypertension and dyslipidemia, lifestyle advice to stop cigarette smoking and to increase physical activity and achieve a healthy weight, are treatment targets at least as important as hyperglycemia.

**Figure 2 | Effects of DPP-4 inhibitors on cardiovascular risk factors in people with type 2 diabetes.**
Adapted with permission from Scheen AJ. Cardiovascular effects of gliptins. *Nat Rev Cardiol.* 2013;10:73-84.51

**Imaging of cardiovascular risk**

The most common underlying process behind CVD is atherosclerosis. Atherosclerosis is a process that develops when plaques appear in the arterial wall, narrowing the arteries. Rupture of a plaque can cause a myocardial infarction or stroke. A plaque prone to rupture is defined as high-risk, or also described as a vulnerable plaque. Several pathological processes such as endothelial dysfunction, inflammation, lipid accumulation, angiogenesis, thrombosis, and calcification are involved in the vulnerable plaque, and may also serve as markers to predict CVD. With conventional morphologic imaging such as B-mode duplex ultrasound and computed tomography (CT) angiography, it is possible to identify stenotic atherosclerotic lesions, but this imaging does not provide any information about underlying vulnerable plaque processes, like arterial inflammation. Nowadays the identification of the vulnerable plaque, with novel imaging modality options, is a hot topic. For example, positron emission tomography (PET) using $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) is a widely used modality for tumor imaging, but is also being used to assess atherosclerosis, and in particular inflammation. In addition, high carotid $^{18}$F-FDG uptake, as a surrogate of arterial
inflammation, predicts CVD independent of traditional risk factors in asymptomatic people. Furthermore, 18F-sodium fluoride (18F-NaF), commonly known as a bone tracer, is a promising marker to assess active, early phase microcalcification. 18F-NaF binds to areas of microcalcification within an (even non-visibility calcified) atherosclerotic plaque. In addition, a clinical study showed that ruptured high-risk coronary plaques have significantly higher 18F-NaF uptake than do low-risk coronary plaques.

Another possibility for imaging of CV risk is quantitative assessment of abdominal adipose tissue by means of Magnetic Resonance Imaging and CT. Visceral abdominal adipose tissue (VAT) is a major contributor to CV risk. It is likely that not only the VAT mass but also the metabolic state of VAT, i.e. overproduction of adipokines, is related to CV risk. Consequently, there is a need for a simple but accurate reproducible tool to analyze abdominal adipose tissue using a 18F-FDG-PET/CT scan.

Finally, other innovative techniques besides imaging modalities may be useful for assessing the CV risk in people with diabetes. An example is applanation tonometry, which can be non-invasively used to assess aortic pulse wave velocity (PWV). PWV is a marker of arterial stiffness and a powerful predictor of CV outcomes in the general population and also in patients with diabetes.

AIMS AND OUTLINE OF THE THESIS

People with type 2 diabetes are at increased risk of CVD because of the interaction of various risk factors. Clearly, hyperglycemia is only one part of the picture for increased CV risk. Moreover, glycemic control is not the only treatment target of type 2 diabetes; managing the CV risk is also an essential target. Obviously, drugs to treat diabetes should, beyond their glycemic effects, also offer CV protection. To evaluate treatment effects beyond only the glycemic effects, there is a considerable need for CV risk markers that may serve as a readout for therapeutic approaches. For the detection of atherosclerosis the well-known CVD risk factors provide less specific information than do novel imaging modalities and innovative techniques. Therefore, assessment of atherosclerosis and CV risk by using imaging modalities and innovative techniques may reveal CV risk markers and provide a readout for therapeutic approaches. Moreover, imaging may uncover the mechanisms whereby diabetes increases CV risk.

The aim of the work presented in this thesis is twofold. Part I focuses on different aspects of CV risk imaging. Chapter 2, as part of the introduction, gives an overview of imaging modalities and different imaging agents to identify pathophysiological processes occurring within the high-risk plaque. Chapter 3 provides more insight into 18F-NaF as a marker of microcalcifications in the atherosclerotic plaque. Chapter 4 presents an evaluation of
the reproducibility and repeatability of a semi-automated method for assessment of the metabolic activity represented as $^{18}$F-FDG uptake of, especially, VAT on $^{18}$F-FDG PET/CT scans.

Part II of the thesis deals with the relationship between different CV markers assessed by means of imaging modalities and innovative techniques, markers representing different stages of atherosclerosis in people with early type 2 diabetes. Chapter 5 discusses the relationship between PWV as a measure of arterial stiffness and arterial $^{18}$F-FDG uptake as a measure of arterial inflammation in people with early type 2 diabetes. Furthermore, part II shows the results of a randomized controlled trial with the DPP-4 inhibitor linagliptin in early type 2 diabetes, investigating treatment effects beyond their glycemic effects. Chapter 6 describes the treatment effects of linagliptin on PWV as a surrogate marker of arterial stiffness and early atherosclerosis. Chapter 7 presents the treatment effects of linagliptin on arterial $^{18}$F-FDG uptake as a measure of arterial inflammation. The final chapter, Chapter 8, summarizes the results of this thesis and discusses future perspectives.
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