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ON MACROVASCULAR AND RENAL COMPLICATIONS IN TYPE 1 DIABETES MELLITUS: SOME ASPECTS ON GLYCEMIC MEMORY

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On macrovacular and renal complications in type 1 diabetes mellitus: some aspects on glycemic memory

Medicinsk avhandling

av

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To all children and adolescents with type 1 diabetes. Time is on your side.

ABSTRACT

There is a substantially increased risk of premature mortality and morbidity in cardiovascular disease (CVD) among type 1 diabetes individuals compared to individuals without diabetes. Development of microangiopathy and markers of macrovascular complications precedes these events. The SDIS and subsequent DCCT/EDIC trials conclusively established already in 1993 that early intensified insulin treatment halts microvascular complications. This has also been shown for macrovascular complications in the DCCT/EDIC trial, a finding suggested to be due to glycemic memory.

In this thesis we first (**Study I**) aimed to investigate early signs of atherosclerosis, measured as carotid intima-media thickness (cIMT), and its relation to insulin sensitivity in young type 1 diabetes individuals.Then we compared (**Study II**) skin microvascular function in the foot and the time to first of hospitalization for ischemic foot ulcer in between the two groups in the SDIS cohort. We also investigated (**Study III**) long-term follow-up complications in the SDIS cohort, comparing incidence in all-cause mortality, mortality in CVD, as well as incidences in CVD events, *i.e.* myocardial infarction and stroke, and diabetic nephropathy, between patients earlier randomized for 7.5 years to intensive insulin treatment *vs.* standard treatment. Finally, we investigated (**Study IV**) long-term survival in individuals with type 1 diabetes, type 2 diabetes and without diabetes following coronary artery bypass grafting (CABG). In this study we combined the SWEDEHEART and the Swedish national diabetes registers. The outcome measures were all-cause mortality, mortality in CVD death and any major adverse coronary event, *i.e.* myocardial infarction, heart failure, stroke or need of revascularization.

In **study I,** young type 1 diabetes individuals had an increased cIMT concomitant with insulin resistance compared to the non-diabetic group. In a multivariate analysis, insulin resistance was significantly associated to an increase in cIMT. In **study II,** 13 patients developed ischemic foot ulcer during the median 28 years of follow-up. Foot skin microcirculation blood flow was higher in the earlier intensively insulin-treated group compared with the standard treated group. Glycemic control measured as HbA_{1c} levels was independently associated with endothelial-dependent vasodilatation and capsaicin-induced vasodilatation. In **study III**, during 28 years of follow-up 22 persons died. There was no significant difference between groups for all-cause mortality, mortality in myocardial infarction, stroke or ESRD, or for morbidity in myocardial infarction or stroke. One person in the ICT group compared with seven in the ST group developed ESRD. HbA_{1c} did not differ between the two groups during the last 16 years of follow-up. In **study IV**, with a mean follow-up time of 5.9 years, a total of 6,765 out of 39,235 patients died: 17 % of whom had no diabetes, 21 % had type 1 diabetes, and 19 % had type 2 diabetes. The risk for all-cause mortality was doubled in type 1 diabetes, compared to type 2 diabetes. The risk of death was similar among type 1 diabetic men and women.

Adolescents with type 1 diabetes show early signs of atherosclerosis compared to a matched control group. This was demonstrated together with insulin resistance. Earlier intensively insulin-treated type 1 individuals from the SDIS trial seem to have a favorable prognosis regarding the development of foot ulcers and diabetic nephropathy, compared to the standard treated individuals. This was demonstrated despite the same glycemic control for the last 16 years in the follow-up. Type 1 diabetes individuals have much poorer outcome after CABG intervention compared to type 2 diabetes individuals.

LIST OF SCIENTIFIC PAPERS

I. Rathsman B, Rosfors S, Sjöholm A, Nyström T. Early signs of atherosclerosis are associated with insulin resistance in non-obese adolescent and young adults with type 1 diabetes. *Cardiovasc Diabetol. 2012 Nov 27;11:145*

II. Rathsman B, Jensen-Urstad K, Nyström T. Intensified insulin treatment is associated with improvement in skin microcirculation and ischaemic foot ulcer in patients with type 1 diabetes mellitus: a long-term follow-up study. *Diabetologia. 2014 Aug;57(8):1703-10*

III. Rathsman B, Donner M, Ursing C, and Nyström T. Intensified insulin treatment decreases the risk of end stage renal disease in type 1 diabetes: a long-term follow-up study. *Manuscript submitted*

IV. Holzmann MJ, Rathsman B, Eliasson B, Kuhl J, Svensson AM, Nyström T, Sartipy U. Long-term Prognosis in Patients with Type 1 and Type 2 Diabetes Mellitus After Coronary Artery Bypass Grafting. *J Am Coll Cardiol 2015;65:1644–52*

CONTENT

LIST OF ABBREVIATIONS

ABI	Ankle-brachial index
ACEI	Angiotensin-converting enzyme inhibitor
ACh	Acetylcholine
AGE	Advanced glycation end product
ARB	Angiotensin receptor blocker
BMI	Body mass index
CABG	Coronary artery bypass graft
CAD	Coronary artery disease
CCA	Common carotid artery
CDR	The Swedish Cause of death register
CHD	Coronary heart disease
CIMA	Carotid intima media area
cIMT	Carotid intima-media thickness
CVD	Cardiovascular disease
eGDR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
GFR	Glomerular Filtration Rate
GIR	Glucose infusion rate
HDL	High density lipoprotein
HR	Hazard ratio
IPR	The Swedish Inpatinet register
LDL	Low density lipoprotein
MACE	Major adverse coronary event
NDR	Swedish national diabetes register
PAD	Peripheral artery disease
PU	Perfusion unit
sBP	Systolic blood pressure
S_i	Insulin sensitivity
SNP	Sodium nitroprusside

1 INTRODUCTION

I sat down with a two year old child and his parents in a small emergency ward room. This was our first talk about type 1 diabetes, but many were to follow. The child's random taken plasma glucose being above 20 mmol/L left no alternative. For me, this was a routine situation. For the parents in front of me, it was a shock and scare. The father asked all the questions I expected - how, why, what will the treatment be? Then the mother turned to me and asked: "What is his life going to be like, is he going to live to be old?" As I remember it, my answer at that time was something about us together doing all we could to make the boy's quality of life as good as possible, and that the future for someone with diabetes was ever changing for the better due to research and advances in treatment. However, after the meeting, alone in my room, her question kept coming back to me. It contained many layers. Life is certainly complicated for a child with diabetes. Many daily considerations are needed, blood tests taken, injections given, meals eaten and frustrations handled. Surprisingly, all this is often very manageable. Of course, it was impossible to give her a detailed answer, but did I really know what to expect? What was I to look for, except optimizing treatment? At what age should I introduce preventive treatments? What do we really know about the development of complications? These questions recurred to me and I realized I needed to know more.

This thesis project started out as a brief discussion between Dr Anna Kernell and me regarding hypertension and the risk of cardiovascular problems for the young type 1 diabetes patients we encountered in our daily work at the pediatric outpatient unit. The idea of evaluating their actual situation, and search for possible signs of development of vascular complications, very slowly progressed into the initiation of the first study. We began by enrolling adolescents from our everyday work, and Dr Kernell and I also visited schools in the southern area of Stockholm to recruit controls. Very sadly, Dr Kernell passed away and left me stumbling with my thesis project. Fortunately my co-supervisors stepped in, and others were recruited, enabling my thesis work to continue.

After finishing the first study I and my co-workers realized the need for experience in the long-term and adult diabetes perspective to come further with our questions. Most fortunately I was introduced to the dedicated work of Dr Per Reichards in the Stockholm Diabetes Intervention study, addressing microvascular complications in type 1 diabetes and the role of glycemic control. I was entrusted with the remains of this landmark study and given the opportunity to trace the participating patients and follow up his data. This process lead to something similar to a detective investigation, with us looking for clues to find data in cardboard boxes stacked away in various units of the hospital.

I could not have done this project without the help from my supervisors. Also, I am in great debt to Dr Kernell and Dr Reichard. I hope this thesis is something they would have appreciated.

1.1 DIABETES

Type 1 diabetes is an autoimmune-mediated or immune-associated destruction of insulin-producing islet β-cells in the pancreas, eventually leading to a loss of insulin secretion [1]. Due to the loss of β-cells, persons developing type 1 diabetes are dependent on exogenous insulin treatment. Even though the disease may develop at any age, the peak incidence is in childhood or in puberty [2]. The incidence of type 1 diabetes varies a lot between countries in the world, with the highest rates in Finland [3]. An increased incidence has been observed in many countries over the last two to three decades [4], though the incidence in Sweden has been stable during the last years [5]. Type 1 diabetes is diagnosed based on typical age, the clinical finding of hyperglycemia, polyuria, polydipsia and weight loss, low fasting C-peptide levels and detection of autoantibodies against glutamic acid decarboxylase 65 (GAD); tyrosine phosphatase-like insulinoma antigen 2 (IA2); insulin (IAA); and β-cell-specific zinc transporter 8 autoantibodies (ZnT8). The diagnosis is strengthened by existence of HLA risk types (most frequent HLA DR3/DR4) [6, 7]. There is also a less frequent type 1 diabetes subtype, type 1 diabetes b, which is also characterized by β-cell destruction but without any evidence for the involvement of autoimmune assault in its etiology [8]. Type 2 diabetes, on the other hand, is by far the most common form of diabetes (accounting for 90-95 % of cases) and mostly affects middle-aged and older patients [9]. The mechanism behind type 2 diabetes is thought to be initiated by the development of insulin resistance, mainly in skeletal muscle and the liver. Usually this is adjusted for by an enhanced insulin secretion by the pancreatic β-cells [9]. However, persons developing type 2 diabetes are unable to compensate the insulin resistance by adequately increasing insulin secretion. Obesity is a major factor inducing insulin resistance and it frequently coexists with type 2 diabetes. The incidence of type 2 diabetes is increasing rapidly worldwide and most probably due to a more sedentary lifestyle with excess caloric intake and decreased exercise, leading to obesity, and to generally older populations in many countries [10]. Type 2 diabetes, in contrast to type 1 diabetes, is also strongly influenced by genetic predisposition.

The diagnosis of diabetes is confirmed by finding random plasma glucose concentration ≥11.1 mmol/L or fasting plasma glucose concentration ≥7.0 mmol/L at two separate occasions. Fasting is defined as no caloric intake for at least 8 h. The diagnosis can also be made by the finding of a two hour postload glucose concentration ≥11.1 mmol/L during an oral glucose tolerance test. A random finding of a non-fasting plasma glucose concentration ≥11.1 mmol/L and classic clinical symptoms is also diagnostic. In 2011 WHO stated that a level of HbA_{1c} >48 mmol/mol at two separate occasions is also diagnostic [11], a definition now also recommended by the American Diabetes Association [7].

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommend the use of mmol/mol as an international standard unit for HbA_{1c} and this method is used in Sweden since 2011. Therefore the mmol/mol unit will be used throughout this thesis. However, HbA_{1c} expressed in % (DCCT standard) is often applied in reports and medical journals and will also, to some extent, be presented in the thesis.

1.2 CARDIOVASCULAR DISEASE AND TYPE 1 DIABETES

The Banting and Best discovery of insulin in 1921 and the subsequent development of better and more sophisticated treatments for type 1 diabetes have dramatically changed not only the prognosis of survival in type 1 diabetes but also the spectrum of complications from acute, *e.g.* diabetes ketoacidosis, to chronic [12]. The long-term complications can be further divided into micro- and macrovascular. The macrovascular complications are mainly divided into coronary heart disease (CHD), ischemic stroke and peripheral artery disease (PAD). A brief exposé of microvascular complications will be presented in section 2.5.10.

Throughout the world, cardiovascular disease (CVD) mortality has declined in the general population [13, 14]. This trend is similar in diabetic patients. Gregg and associates analyzed the incidence of acute myocardial infarction, stroke, lower leg amputation, nephropathy and death in hypoglycemic crisis in the United States (between 1990 and 2010), comparing diabetic individuals with the background population. For acute myocardial infarction there were 96 fewer cases per 10,000 persons per year, and 60, 30 and 8 fewer cases per 10,000 persons per year for stroke, lower-extremity amputation and end-stage renal disease (ESRD), respectively. One limitation of this study is that it does not discriminate between the two major diabetes types [15]. Despite the lower incidence of CVD in diabetic individuals, type 1 diabetes is still associated with an increased risk of CVD [16], which in turn is the most common cause of death in this patient group [17].

Diabetes is also associated with a shorter life-expectancy [18]. In the Allegheny County, Pennsylvania, registry of type 1 diabetes individuals, mortality rate after 30 years of diabetes was demonstrated to be six times higher than in the general population [19]. In the Pittsburgh Epidemiology Diabetes Centre (EDC) study, two sub-cohorts, *i.e.* diabetes diagnosis between 1950-1964 and 1965-1980, respectively, were compared, demonstrating life-expectancy to be approximately 15 years longer for the latter group [18]. This clearly demonstrates a substantial progress in avoiding complications, *e.g.* improvement in type 1 diabetes therapy. On the other hand, a group in Scotland recently showed, in a nationwide prospective cohort study of type 1 diabetes individuals, that life-expectancy, from age 20 years, was 11 and 13 year shorter, in men and women, respectively, compared to non-diabetic individuals [20]. The excess mortality in type 1 diabetes individuals was also recently demonstrated in a Swedish nationwide register-based study including more than 30,000 type 1 diabetes individuals, revealing a doubled risk of death (all-cause or from CVD) despite good glycemic control compared to a matched control group [21], a risk substantially increased with increased levels of HbA_{1c} [21].

In the 30 year follow-up part of the Diabetes Control and Complication Trial (DCCT), *i.e.* the Epidemiology of Diabetes Interventions and Complications (EDIC) study, it was again demonstrated that the highest proportion of deaths was attributed to CVD [22]. Even though evidence points towards a decline in mortality and CVD mortality incidence in diabetes, there is still a substantially increased risk of CVD mortality in type 1 diabetes individuals [19, 20, 23].

1.2.1 Atherosclerosis

Atherosclerosis is the primary cause of stroke and CHD, contributing to about 50% of all the deaths in the western countries [24]. Macrovascular complications begin with atherosclerosis, a chronic and multifactorial disease associated with a wide range of independent risk factors, including traditional risk factors such as smoking, hypertension, dyslipidemia, increasing age, family history, obesity and diabetes [25, 26].

Atherosclerosis is considered an inflammatory disease and the first observed lesion is the formation of fatty streaks, *i.e.* mainly macrophages and T-lymphocytes, within the vessel wall of large and middle size arteries. These early changes have been observed already in children and adolescents [27]. The current hypothesis of atherosclerosis development points out endothelial dysfunction to be an early event and the result of injury caused by *e.g.* dyslipidemia, hypertension, smoking, diabetes, infections or genetic factors. Low-density lipoprotein (LDL) cholesterol, modified by for example oxidation or glycation (in diabetes), is today considered the major cause of endothelial injury. The injury causes increased endothelial permeability and adhesiveness of leukocytes and platelets, as well as a pro-coagulation state [25, 26]. The subsequent event is migration and proliferation of smooth muscle cells leading to thickening of the artery wall and, as the inflammation proceeds, more macrophages and lymphocytes are recruited to the lesion. They cause a local activation of cytokines, chemokines, hydrolytic enzymes and growth factors eventually leading to necrosis and formation of a fibrous cap protruding into the vessel lumen. The artery can, to some extent, compensate these events by dilation but at some point the blood flow may be affected causing distal ischemia [25]. However, the fibrous cap may rapidly rupture leading to formation of a thrombosis and, for example, if it involves coronary arteries or brain arteries, an acute coronary syndrome or an ischemic stroke, respectively.

A large number of studies have conclusively demonstrated that individuals with type 1 diabetes have an early onset and a higher degree of atherosclerosis compared to a non-diabetic population. It is typically also more disseminated and aggressive as compared to non-diabetic individuals. Type 1 diabetes patients therefore carry an additionally higher risk of CVD events [28]. Also, type 1 diabetes individuals have a higher frequency of hypertension, dyslipidemia and nephropathy, all contributing to the increased risk of CVD [29-31].

2 EPIDEMIOLOGY OF MACROVASCULAR DISEASE IN TYPE 1 DIABETES

First to investigate CVD in a population-based study was the Framingham Heart study. This study was initiated in 1948 as a community-based prospective cohort study including more than 50,000 adult individuals from the general population. Participants underwent physical examination and laboratory tests every other year and were followed up regarding all-cause mortality, CVD mortality and morbidity, and associated risk factors [32]. In 1971, children of couples participating in the study and their spouses were invited to the Framingham Offspring Cohort Study. They also underwent physical examination and laboratory tests, as well as ECG every four years [33]. The Framingham study clearly demonstrated that diabetes (mainly type 2) is a major risk factor of developing CVD regardless of gender [34]. However,

it was later confirmed by others that women with diabetes carry an even higher risk of developing CVD [35].

The Pittsburgh EDC study recruited insulin-dependent diabetes patients under the age of 17 at disease onset, and within one year after the diagnosis of diabetes [36]. The individuals were followed-up every second year regarding the development of nephropathy, retinopathy, neuropathy and macrovascular disease (*i.e.* PAD, CHD and stroke). The 10-year follow-up results demonstrated that 10 % of the participants developed CVD during 30 years of diabetes [36]. There were no significant gender differences in the outcomes, except for PAD demonstrating a three-fold higher incidence rate in women after 25 years [36].

In the EURODIAB cross sectional complication study, more than 3,000 type 1 diabetes individuals from 14 European countries, with an age of 15-59 years old, were enrolled. The prevalence of CVD in this study was shown to be 25 % in the group aged 45-59 years, whereas the diabetes duration also had a great impact on CVD prevalence: 15 % and 13 % with diabetes duration of ≥15 years, in men and women, respectively [37].

In the Diabetes UK cohort study 23,751 patients with insulin-treated diabetes diagnosed under the age of 30 years were recruited between 1972 and 1993. Primary outcome was mortality from CHD, in which 1,437 deaths were registered during a mean follow-up of 17 years. CHD in men accounted for 8 % of deaths in the age 20-39, being increased to 47 % in age 40-84 [38]. Roughly the same incidence of CHD mortality was observed in women, 11 % *vs.* 40 % in the same ages [38]. Most notable was that the absolute excess risk of dying from CHD in the age groups 20-29 and 30-39 years was the same for women and men; however, the standardized mortality ratio (SMR) for women was 45 and 42 in those age groups, respectively, compared to the general female population [38].

In the UK General Practice Research database (GPRD) with 7,479 type 1 diabetes patients, CVD events developed 10 to15 years earlier compared to the general population [16]. In this study, CVD events were significantly higher in women compared to men [16].

As early as in the Framingham cohort 20 years follow-up study, CVD was demonstrated to be six times more prevalent in diabetic women aged 45-54 years compared to a matched population [12]. Over the years, studies have yielded conflicting results; with less risk for women [39], increased risk [38, 40, 41], or relatively the same risk [42], as for men. A recent meta-analysis, including more than 200,000 individuals with type 1 diabetes, demonstrated a 40 % greater relative risk of all-cause mortality and more than doubled the risk for CHD in women, compared to men [43].

In the context of epidemiological cohort studies of CVD in type 1 diabetes individuals one needs to mention the DCCT and the follow-up EDIC study. Recently the DCCT/EDIC writing group demonstrated a similar pattern with a decrease for all-cause mortality in the intensive insulin treatment group, the highest proportion of deaths (20%) were attributed to CVD [22]. The DCCT study and the contemporary Stockholm Diabetes Intervention Study (SDIS) were landmark studies demonstrating the benefits of improving glycemic control to lower the incidence of microvascular complications [44, 45]. They will be thoroughly discussed later on in this thesis.

2.1 CORONARY HEART DISEASE

Diagnosis of CHD in diabetes may be challenging. Myocardial ischemia is often silent in diabetes patients due to autonomic and somatic sensory neuropathy. The majority of myocardial infarctions may be asymptomatic [46], or presenting in a highly atypical manner (*e.g.* syncope, sudden shortness of breath or nausea), and therefore diagnosed much later by electrocardiogram (ECG) screening, or with myocardial scintigraphy or stress echocardiography.

Over the years, mounting evidence has been supporting an increased risk of CHD in type 1 diabetes individuals [38, 47, 48]. Among studies reporting the incidence of CVD in type 1 diabetes, CHD consistently stands out as the most common [16, 20, 49, 50]. In the UK GPRD study, the relative risk of acute CHD events was demonstrated to be 3.0 (95% CI 2.2–4.1) for men, and 7.6 (95% CI 4.9–12.0) for women compared to non-diabetics [16]. Age-adjusted cumulative incidence of CHD in type 1 diabetes individuals differs between studies, but the average cumulative incidence seems to be around 15 % during a 15-20-year follow-up period [49]. This high incidence rate of CHD was also reported in the Diabetes UK cohort study, in which nearly 25 % of the all-cause mortality was attributed to CHD [16]. The age-specific mortality data from the UK GPRD study showed that the SMR for CHD, compared to the general population, was highly elevated. Most striking was the increased SMR for CHD in women (all ages) compared to men [38].

2.2 STROKE

Stroke is generally divided into two subtypes, *i.e.* ischemic and hemorrhagic stroke. Hypertension and proteinuria are believed to be the most important risk factors for development of stroke [51, 52]. The clinical evaluation is important and stroke scales like National Institute of Health Stroke Scale (NIHSS) are useful tools [53]. The NIHSS is based on eleven parameters ranging from level of consciousness, visual field and eye movement tests to motor function, sensory function, speech, language and attention testes. Each area renders a score from 0 to 4 and the resulting sum defines stroke severity from no stroke to severe stroke and also predicts likelihood of recovery [54]. The diagnosis of stroke is usually confirmed by the use of MRI or CT scanning, demonstrating thrombotic lesions (ischemia) or bleeding.

The incidence of premature morbidity and mortality in stroke is markedly increased in type 1 diabetes patients [55, 56]. This risk is further increased in case of hypertension or proteinuria [57]. It was recently demonstrated in a Swedish registry study that middle-aged (<50 years old) individuals with type 1 diabetes were shown to have a considerably higher risk of stroke as compared to the general population, with even a higher risk for women [55]. The incidence rates were excessively elevated if the patients also had diabetic nephropathy or retinopathy [55]. The incidence of stroke was also investigated in the Finnish Diabetic Nephropathy (FinnDiane) study [58]. The major findings were that diabetes duration, diabetes nephropathy, poor glycemic control, higher systolic blood pressure (sBP), history of smoking and indirect measures of insulin resistance, *i.e.* estimated glucose disposal rate (eGDR), were all independent risk factors of ischemic stroke. However, risk factors partly differed from that of hemorrhagic stroke.

The only other study looking at independent risk factors for stroke in type 1 diabetes is the Pittsburgh EDC study [59]. It was found that diabetes duration, sBP, low levels of high-density lipoprotein (HDL) cholesterol and diabetic nephropathy were strong risk factors for ischemic stroke. However, sBP was confounded as a risk factor by nephropathy in that study [59].

In the 27-year DCCT/EDIC follow-up study all-cause mortality risk was moderately, albeit significantly, lower in the previously intensively treated group [22]. The major cause of death in this long-term follow-up study was CVD with no discrimination between CHD and stroke [22]. There was an association between overall mortality and mean HbA_{1c} level during the 6.5 year in DCCT, demonstrating glycemic control as an important factor [22]. This was also pointed out in the FinnDiane study, *i.e.* poor glycemic control is a risk factor for ischemic stroke [58]. These studies indicate a beneficial role of good glycemic control for the risk of suffering an ischemic stroke.

2.3 PERIPHERAL ARTERY DISEASE

The definition of PAD currently used by the European Society of Cardiology guidelines includes atherosclerotic lesions in the extra-cranial carotid and vertebral, upper and lower extremity, mesenteric and renal arteries [60]. These central atherosclerotic changes are often accompanied by lesions located distally in patients with diabetes, most often in the popliteal artery or in the vessels of the lower leg. The diagnosis of PAD is based on typical symptoms of intermittent claudication and clinical investigations, including auscultation and palpation of peripheral arterial pulses. Ankle-brachial index (ABI) is an objective measurement of peripheral artery disease in the lower extremity and is calculated by dividing the systolic blood pressure at the tibial or dorsalis pedal arteries level with the brachial systolic pressure. An ABI index of <0.8 or a mean of three ABIs <0.9 is diagnostic for lower extremity artery disease, and an index of <0.5 indicates high risk of amputation [60].

Diabetes-related foot disease is a major cause of morbidity and mortality [61, 62]. The reported rates in lower extremity amputations vary from 2.8 to 43.9/100,000/year between countries in the world, and 25-90 % of these are associated to diabetes [62]. It is estimated that about 5 % of all diabetes patients have had a history of foot ulcers [63]. The life-time risk of a diabetes-related foot ulcer is reported to be as high as 25 % [64]. There is a clear association between peripheral neuropathy and foot ulceration [65]. A comparative study of PAD in diabetic and non-diabetic patients confirmed that diabetic patients had more distal disease and a poorer outcome with respect to amputation and mortality [66]. Other reported contributing risk factors in the development of foot ulcers are foot deformities, micro-vascular complications, increase in plantar foot pressure due to deformities, infection and diabetes duration [67]. The ultimate consequence of PAD and foot ulcers is the need for lower-extremity amputation. Some later studies point toward a decrease over time in the incidence of amputations. A Scottish nation-wide study demonstrated a 30 % decrease in the incidence of lower extremity amputations in diabetes patients from 2004-2008 [68]. Also, from the Steno Diabetes Center in Denmark, it was recently demonstrated that the incidence

of amputations have decreased significantly during the last decade [69]. This is suggested to be due to implementing a multidisciplinary team-work [68].

2.4 DIABETES NEPHROPATHY

Diabetes-related kidney disease is graded into microalbuminuria, macroalbuminuria, or ESRD and/or categorized according to the extent of impairment in glomerular filtration rate (GFR). Microalbuminuria is defined as an albumin excretion rate of 30-299 mg/24 h (alt. 20-200 μg/min), whereas macroalbuminuria is defined as an albumin excretion rate of ≥300 mg/24 h (≥200 μg/min) [70]. It should be noted that the definition of diabetic nephropathy includes macroalbuminuria and ESRD, but not microalbuminuria.

The incidence of diabetic nephropathy has been investigated in many cohorts over the years. In the Steno epidemiological study, it was reported that 41 % of the individuals developed diabetic nephropathy [29]. The prevalence was similar in the Pittsburg EDC 10 year follow-up study, showing that 40 % of the individuals developed diabetic nephropathy during 30 years of diabetes duration [36]. In the Steno study, the incidence in proteinuria among patients diagnosed from 1933-1942 and from 1953-1962 was investigated. Findings revealed a 30 % decreased incidence of proteinuria in the younger population compared to the older group; this clearly (and perhaps not so surprisingly) indicates that diabetes duration is an important risk factor for developing diabetic nephropathy [29].

Progression in the extent of diabetic nephropathy has been shown to correlate to an elevated risk of mortality in several studies [71-74]. In the FinnDiane study, an independent graded association was observed between the severity of kidney failure and mortality [72, 75]. However, absence of albuminuria in type 1 diabetes individuals seems to be protective so that the mortality rate is similar to the general population [72, 75].

The impact of glycemic control on the risk of developing renal disease is paramount; lowering HbA_{1c} levels is associated with less albuminuria [45] [44]. It was recently shown in a Swedish followed-up cohort study of type 1 diabetes patients from diagnosis that 23 % of the individuals with long-term (20-24 years) mean HbA_{1c} of >80 mmol/mol developed diabetic nephropathy, whereas none with HbA_{1c} <60 mmol/mol did [76].

Longevity in diabetes is substantially decreased with lower eGFR [20]. On the other hand, there is still a shorter survival for those with type 1 diabetes in the presence of a normal eGFR (≥90 mL/min/1.73 m^2), indicating that factors other than solely nepropathy influence the risk of premature development of CVD and death [20]. The association between loss of kidney function and premature death *per se* and CVD has been hypothesized to be due to that the degree of functional renal impairment reflects a general atherosclerotic development and severity [77]. On the other hand, a long list of other factors (all involved in CVD) is associated with declining renal function, such as male sex, hypertension and dyslipidemia [78], as well as oxidative stress [79], endothelial dysfunction [80], advanced glycemic end products (AGEs) [81], left ventricular hypertrophy [82], and insulin resistance [83]. Good glycemic control and strict blood pressure control are probably the two most important factors decreasing the risk of diabetic nephropathy [84].

2.5 CVD RISK FACTORS IN TYPE 1 DIABETES

Many factors are associated with an increased risk of CVD in type 1 diabetes, figure 1. Some are non-modifiable, such as age, diabetes duration and heredity; whereas others, such as smoking, hypertension, lipid levels, obesity, albuminuria and glycemic control, are modifiable. Most of these factors are discussed in the different sections but will be more thoroughly scrutinized in the following part.

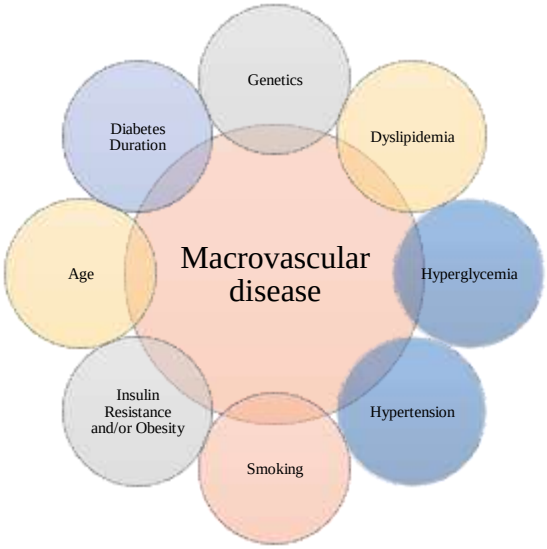

Figure 1 Factors contributing to development of Cardiovascular Disease in type 1 diabetes

2.5.1 Genetics

A growing body of evidence points to different genetic variations being associated with the heightened risk of CVD in type 1 diabetes. One example is the haptoglobin 2-2 (Hp2) genotype, which has been associated with both subclinical atherosclerosis and increased risk of CHD [85, 86]. There are three possible haptoglobin genotypes: 1-1, 2-1 and 2-2, of which Hp2 possesses less antioxidant capacity due to its larger molecular size and the lower oxidative capacity may reduce reverse cholesterol transport via altered modification of apolipoprotein A-I [87].

2.5.2 Smoking

Cigarette smoking is closely coupled to the increased risk of developing not only microvascular [88] but also macrovascular complications [89, 90] in type 1 diabetes. As regards macrovascular complications, the association is strongest for the risk of contracting PAD. The negative effect of smoking is probably due to endothelial dysfunction and inflammation [91].

2.5.3 Hyperglycemia

Brownlee has proposed a model connecting hyperglycemia and oxidative stress with atherosclerosis [92]. In this model, the increases in glycolysis and tricarboxylic acid cycle activity (driven by hyperglycemia) generate an overproduction of superoxide in the mitochondrial electron-transport chain in the endothelial cells [92]. This excess superoxide production is proposed to cause an inhibition of glycolytic enzymes, resulting in an

accumulation of metabolites and cell damage in the blood vessels. In addition, intracellular hyperglycemia is hypothesized to increase the osmotic pressure and vascular permeability [92]. This would allow lipoproteins, such as LDL-cholesterol, to transmigrate into the vessel, accompanied by pro-inflammatory cells, *e.g.* monocytes [25].

AGEs can be formed via oxidation of glucose and lipids and through the polyol pathway [93]. Patients with diabetes have been shown to exhibit increased amounts of tissue AGEs compared to non-diabetics [94]. Increased skin AGE levels are predictive for microvascular complications in type 1 diabetes [95], indicating a connection between hyperglycemia and AGE concentrations. Measurements of skin collagen AGEs from subjects in the DCCT have demonstrated AGE levels to be closely associated to the development of microvascular complications [81].

A strong association between chronic hyperglycemia, *e.g.* measured as HbA_{1c}, and microvascular complications has been unequivocally established in several large epidemiological studies [96-98]. In contrast, the importance of glycemic control in the development of CHD has been very much debated and remains a controversial issue. Some data suggest a relationship [99], while some do not [37, 100]. Others have demonstrated an important role of hyperglycemia in the pathogeneses of stroke and myocardial infarction [38, 97, 101, 102], in which chronic hyperglycemia is associated with CVD, independent of age and other classic risk factors [103]. It has been demonstrated (in large epidemiological studies) that every one percent increase in HbA_{1c} above the upper normal range is associated with a 20-30 % increase in CVD events [103]. However, not all studies have arrived at the same conclusions. The EURODIAB study failed to demonstrate any association between HbA_{1c} levels and CHD in women but did so in men [104]; neither did the Pittsburgh EDC 10 year follow-up study show any association between glycemic control and CVD events [105]. A comparison between the two cohorts revealed no association between HbA_{1c} levels and CHD in men but did so among women [106]. Moreover, a later report from the Pittsburgh EDC study demonstrated that hyperglycemia aggravated the CHD risk even more if albuminuria was also present [107]. The DCCT/EDIC study also conclusively and unequivocally established that strict glycemic control is one major factor in modifying the risk of all-cause mortality and CVD in the long-term perspective [22].

According to the current guidelines from European Association for the Study of Diabetes (EASD) and the American Association of Diabetes (ADA), the target HbA_{1c} level -- in terms of preventing vascular complications -- is <52 mmol/mol [108], a level also recommended by the Swedish National Board of Health and Welfare (Socialstyrelsen). However, in select diabetic patients, *e.g.* elderly with short expected life expectancy and patients with long diabetes duration with previous CVD events, (particularly type 2 diabetes individuals), the over-arching goal may, or even should, not be to strive for achieve normal HbA_{1c} levels [109].

2.5.4 Dyslipidemia

Generally, lipid levels (and their subfractions) seem to be of about the same magnitude in adult type 1 diabetics with good glycemic control as in non-diabetic individuals [110]. On the other hand, studies have demonstrated higher lipid levels in adolescent and young adults with

type 1 diabetes compared to matched control individuals [31]. Good glycemic control can to some extent improve or even normalize certain components of diabetic dyslipidemia, especially hypertriglyceridemia [111]. Other established factors contributing to higher lipid levels are BMI and insulin resistance [112]. In the Framingham offspring study (general population) low HDL-cholesterol, high LDL-cholesterol and high triglyceride levels were found to be associated with an increased risk of CVD [113]. In the Coronary Artery Calcification in Type 1 diabetes (CACTI) study, elevated fasting levels of triglycerides were independently associated with increased coronary artery calcification progression [114]. Low HDL-cholesterol levels in individuals with type 1 diabetes are associated with an increased risk of CHD [115]. In the Pittsburgh EDC study, LDL-cholesterol levels >2.6 mmol/L were found to be associated with an increased CVD risk [116]. Whereas it is generally agreed that the utility of lowering LDL-cholesterol levels in type 2 diabetes patients nowadays is undisputed [117], controversy lingers as to whether this is also the case in type 1 diabetes. However, treatment, chiefly by statins, indicates that LDL-lowering can be beneficial in reducing CVD incidence also in patients with type 1 diabetes [118, 119].

2.5.5 Hypertension

Hypertension is a common and over-represented co-morbidity among both type 1 and type 2 diabetes patients [30, 120], compared to the general population, and is a strong predictor of CVD events [106]. Both the ADA and the EASD recommend a blood pressure goal of <130/80 mmHg for diabetes individuals [121, 122]. The CACTI study demonstrated that only 42% reached the blood pressure goal (<130/80 mmHg) set for adults [123]. In the SEARCH Diabetes in Youth Study, the prevalence of hypertension was 6 % and the prevalence increased in obese adolescents and individuals with poor glycemic control [124]. There is a clear association between hypertension and poor glycemic control as well as diabetic nephropathy [120, 125]. This was demonstrated in, among other studies, the DCCT/EDIC in which higher HbA_{1c} levels were associated with hypertension; in this study intensive insulin replacement therapy was also found to decrease the risk of incident hypertension [126]. It might be speculated as to whether, or to what extent, this is due to insulin's vasodilatory features. In type 1 diabetes, hypertension is most often treated with ACEi or ARBs, oftentimes with low-dose thiazide diuretics or calcium channel antagonists as add-on therapy, if deemed necessary. It is particularly important to strive for rigorous blood pressure control if the type 1 diabetes patient also has albuminuria.

2.5.6 Insulin resistance

Increased risk for diabetes-related vascular complications is associated with components of the metabolic syndrome, in which insulin resistance is an important factor. This has been conclusively shown particularly in type 2 diabetes individuals [74, 127]. The Pittsburgh EDC used eGDR as a surrogate marker for insulin resistance, and attempted to investigate the relation between this marker and the risk of incident CHD. They demonstrated eGDR to be a predictor, but not glycemia, of CHD in type 1 diabetes [105, 128]. In the DCCT/EDIC study, patients with the highest degree of insulin resistance, as estimated by eGDR at baseline, were found to have the highest risk of developing both micro- and macrovascular complications, irrespective of which treatment group they were randomized to. This gives some clues, or at least hints, that insulin resistance *per se* may be an important risk factor for diabetic

angiopathy not only in type 2 diabetes but also in type 1 diabetes [129]. It has been proposed, in a mathematical model, that prevention of insulin resistance would lower rates of myocardial infarction as much as 40 % regardless of the other risk factors involved in the metabolic syndrome [130].

The quantitative impact of insulin resistance on CVD is not easy to dissect out, since it is co-incident with several other traditional CVD risk factors, *e.g.* hypertension, obesity, and dyslipidemia [130]. Nonetheless, some epidemiologic studies lend support to the notion that insulin resistance is pathogenically important for CVD development also in type 1 diabetes. In the Pittsburgh EDC study, the hazard ratio of CHD was increased for those with a family history of type 2 diabetes, even after adjustments for known confounding factors in the insulin resistance syndrome [131]. The term "double diabetes" has been coined to describe the phenomenon that type 1 diabetes patients with family history of type 2 diabetes often-times are also overweight and have sub-optimal glycemic control despite high insulin doses [132]. Theoretically, it has been debated if these findings and the autoimmune development of type 1 diabetes are two independent processes, or if insulin resistance triggers or facilitates/accelerates the autoimmune process [133].

Excessive weight gain, not infrequently occurring as a direct consequence of intensified insulin treatment, has been considered as a cause for concern of contributing to CVD events in type 1 diabetes [134]. This was addressed among adult DCCT patients where it was found that the quartiles who gained most weight in both treatment groups also had the highest blood pressure and lipid levels [134]. A later study confirmed that markers of macro-vascular complications were significantly correlated with weight gain (both in the intensive and conventional treatment groups) [135]. This also forms part of the rationale for the clinical practice of sometimes treating obese type 1 diabetes patients, albeit 'off-label', with metformin as add-on therapy to insulin. On the other hand, a recent publication from the DCCT/EDIC group report consistent lower all-cause and CVD mortality in the group previously intensively treated with insulin [22].

2.5.7 Diabetes nephropathy

Diabetes nephropathy is strongly associated to CVD. The Steno-2 study clearly demonstrated that the risk of CVD was four times higher in type 1 diabetes individuals with micro-albuminuria, as compared to those without [136]. Macroalbuminuria renders the diabetes patients susceptible to developing CVD. In the FinnDiane study, as well as in the Pittsburgh EDC study, both micro- and macroalbuminuria and ESRD correlated significantly with increasing mortality risk for each stage of renal compromise [72, 75].

Conversely, it is important to keep in mind that an improvement in glycemic control reduces the incidence of microalbuminuria. This was firmly demonstrated in the SDIS and slightly later confirmed in the DCCT in 1993 [44, 45]. The continuing DCCT/EDIC study has confirmed these findings and also demonstrated that improvement in glycemic control and hypertension may reverse the degree of albuminuria [84].

2.5.8 Risk factors in childhood and adolescence

Both the ADA and the International Society for Pediatric and Adolescent Diabetes (ISPAD) have recently published guidelines addressing CVD risk factors in children and adolescents with diabetes [137, 138]. They both state that major risk factors for CVD include hypertension, dyslipidemia and hyperglycemia. Traditionally, they have been differing in goals set for HbA_{1c}, with the ADA giving age-specific targets of 70 mmol/mol (DCCT 8.5%) <6 years of age, 64 mmol/mol (DCCT 8%) between 6–12 years of age and 58 mmol/mol (DCCT 7.5%) between 13–18 years of age and the ISPAD recommendation is setting a target of 58 mmol/mol (DCCT <7.5%) for all children with diabetes mellitus. Recently, ADA has adopted the ISPAD recommendation as well.

Management approaches for dyslipidemia are the same between the organizations, *i.e.* lifestyle changes (diet and exercise) and statin use if LDL-cholesterol levels exceed 2.6 mmol/L. Similar approaches apply for the treatment of hypertension, *i.e.* lifestyle changes and ACEi or ARBs if blood pressure > 95^{th} percentile for age and height. The guidelines also emphasize smoking avoidance/cessation, physical activity promotion and treatment of obesity as important interventions. Hypertension is quite common in children and adolescents with type 1 diabetes [124], in whom obesity, hyperglycemia and diabetic nephropathy aggravate this situation [139]. These and other studies make it clear that elevated traditional CVD risk factors, such as high levels of HbA_{1c}, blood pressure and cholesterol, not only are all quite common during childhood and adolescence of patients with type 1 diabetes [140], but also that surprisingly few of the patients receive antihypertensive or lipid-lowering medications, in spite of current guidelines from both the ADA, EASD and ISPAD.

2.5.9 Hypoglycemia

Hemodynamic changes evoked by hypoglycemia include increased heart rate, widening of pulse pressure, due to reduced central blood pressure and peripheral artery resistance, as well as increased peripheral blood pressure [141]. Increased stroke volume and cardiac output, in synergy with these changes, may cause a temporary and abnormal workload of the heart which may be extra harmful for type 1 diabetes individuals with a propensity for CVD that may has hitherto remained asymptomatic/subclinical [141, 142].

Hypoglycemia is also assumed to cause malignant arrhythmias by prolonging the QT interval and cardiac repolarization, thereby increasing the risk of sudden death. In one autopsy study, it was demonstrated that sudden death was four times more common in type 1 diabetics compared to non-diabetic persons [143]. It is tempting to speculate that counter-regulatory hormonal response to hypoglycemia, not least including excessive release of catecholamine's (*e.g.* epinephrine and norepinephrine) and other 'stress hormones', may elicit deleterious effects on the diabetic heart by for instance increasing heart rate and oxygen demand and restricting coronary blood flow.

Type 1 diabetes individuals are at great risk of iatrogenic hypoglycemia, not least due to the fact that insulin resistance is much less of a problem in type 1 diabetes as most of these patients are non-obese. Both in the DCCT and the SDIS studies, an increased incidence of severe hypoglycemia in the intensively insulin-treated groups was demonstrated [44, 45]. Notwithstanding this, none of these studies was designed to address long-term CVD

consequences of severe hypoglycemia. In a Swedish registry study, 1,839 type 1 diabetes subjects with CVD complications were investigated. Of these subjects, 22 % had a history of previous hospitalization for severe hypoglycemia. Further a dose-response relationship between the numbers of previous hypoglycemia and survival after CVD, as well as CVD mortality, was demonstrated [144].

2.5.10 Microvascular complications

The present thesis project has its focus on macrovascular complications of diabetes. On the other hand, study II has a combined micro- and macrovascular approach with chronic foot ulcers as the primary outcome measure. The development of diabetic foot ulcers usually starts with neuropathy, but is also closely related to the development of PAD. Therefore, microvascular complications, including diabetic nephropathy, are also discussed with the attempts to address possible mutual mechanisms between micro- and macrovascular complications in type 1 diabetes.

The long-term microvascular diabetic complications are traditionally divided into retinopathy, neuropathy and nephropathy. In the Pittsburgh EDC study 10-year follow-up, results demonstrated that almost all participants developed mild retinopathy during the first 20 years after diagnosis and -- by 30 years of disease duration -- almost 70% developed proliferative retinopathy [36]. Moreover, in the same study, some 70 % of the participants developed peripheral neuropathy. In the 25-year follow-up of Wisconsin Epidemiologic Study of Diabetic Retinopathy, the overall incidence of any retinopathy was as high as 97 %, with a cumulative incidence of progression to proliferative retinopathy of 42%. Over the years of follow-up, the level of glycemia -- both at baseline and at the follow-up study years -- was closely related to the progression of retinopathy [145].

A strong association between chronic hyperglycemia and microvascular complications has been conclusively demonstrated in several large epidemiological studies [96-98]. As mentioned above, the SDIS and -- slightly later -- the DCCT trials were both pioneering and groundbreaking studies demonstrating that intensified insulin treatment halts microvascular complications in type 1 diabetes due to improved glycemic control [44, 45].

This treatment had also long-term beneficial effects on the macrovascular complications [101]. Whether microvascular dysfunction precedes the development of macrovascular complications remains a controversial and elusive issue. However, microvascular complications in type 1 diabetes, such as peripheral neuropathy [146], nephropathy [147] and retinopathy [148], are all independent risk factors for macrovascular disease.

2.6 TREATMENT OF MODIFIABLE RISK FACTORS IN TYPE 1 DIABETES

The DCCT and SDIS trials both found an undisputable effect on diminishing microvascular complications by means of intensive insulin treatment and thereby improved glycemic control [44, 45]. The DCCT/EDIC study also lends robust support to the view that early and intensive insulin replacement therapy is a major beneficial factor in alleviating the risk of microvascular complications , and of all-cause mortality and CVD in the long-term perspective [22]. A similar pattern has been demonstrated for type 2 diabetes in the UK

Prospective Diabetes Study (UKPDS), with firm effects on microvascular complications, and later on evidence for CVD risk reduction [149].

2.7 DIABETES CONTROL AND COMPLICATION TRIAL/EPIDEMIOLOGY OF DIABETES INTERVENTIONS AND COMPLICATIONS (DCCT/EDIC)

In the 1960´s, the standard treatment regimen of type 1 diabetes was a single morning dose of long-acting insulin. Self-monitoring of blood glucose levels did not exist; urinary glucose excretion was sometimes monitored. Sometimes short-acting insulin was introduced as well. During the 1970´s and more so in the 1980´s, the frequency of insulin doses increased with the introduction of pen-injectors and the first insulin pumps and so the number of short-acting insulin injections, needed to curb prandial glucose excursions, per day increased. This, in conjunction with the establishment of HbA_{1c} measurements and blood glucose self-monitoring, made it possible to improve glycemic control more easily.

The DCCT was launched in 1983, eventually enrolling 1,441 patients with type 1 diabetes. They were randomly assigned to receive either intensified anti-hyperglycemic treatment or standard treatment with a mean follow-up of 6.5 years and evaluated with respect to incidence and development of microvascular complications, *i.e.* retinopathy, nephropathy and neuropathy [150]. Patients in the intensively treated group were introduced to three or more insulin injections per day -- or insulin pump treatment -- in contrast to the conventional treatment group which had one or two daily insulin injections. Glycemic goal was to achieve blood glucose levels as close to normal as possible. Out of the 1,441 included patients 726 were included in a primary prevention (no retinopathy, urinary albumin excretion rate <40 mg/24 hrs), while the remaining 715 in a secondary prevention (mild or non-proliferative retinopathy and urinary albumin excretion rate <200 mg/24 hrs) cohort [150].

The outcome of this landmark study showed a significant lowering of HbA_{1c} levels in the intensively treated group as compared to the control group (55 mmol/mol *vs.* 76 mmol/mol IFCC standard [corresponding to 7.2 % *vs.* 9.1 % DCCT standard]), accompanied by a relative risk reduction of 76 % (primary prevention) and 54 % (secondary prevention) regarding the progression rate of retinopathy. The relative risk reduction for progression of peripheral neuropathy was 60 % and 54 % for nephropathy [96]. These very impressive improvements were, however (and perhaps not so surprisingly), accompanied by an increased risk of severe hypoglycemia in the intensive treatment group (62 *vs.* 19 episodes per 100 patient years) [96].

A later analysis, at study entry, in DCCT subjects (age 13 to 17 years) demonstrated in the primary prevention cohort that intensive insulin treatment decreased the relative risk of retinopathy by 53 %, while in the secondary prevention cohort the relative risk reduction for progression in retinopathy was 70 % [151]. In this sub-study with adolescent patients, the risk of severe hypoglycemia was three times higher in the intensive insulin therapy group. Another report showed that levels of HbA_{1c}, both at study entry and during DCCT completion, were positively correlated to the risk of retinopathy progression [152]. The first evaluation of macrovascular events demonstrated no statistically significant differences, although the absolute numbers were higher in the conventional treatment group [153]. Risk factors for CVD, such as dyslipidemia were improved in the intensive treatment group, but -- as expected -- weight gain was also significantly greater.

The EDIC study was a multicenter observational study designed to follow up the DCCT cohort [154], and 96 % of the original DCCT patients agreed to participate. After DCCT was ended, all patients were offered the intensive treatment regimen. As a result, two years later, 95 % of the former intensive treatment group and 69 % of the conventional treatment group were treated according to the intensive treatment regimen. Despite this, HbA_{1c} levels converged between the groups and were no longer statistically significantly different between the two groups over the five years of EDIC [155], and have been remained similar with a mean HbA_{1c} level of approximately 64 mmol/mol for both groups [156].

As a marker of atherosclerosis, cIMT was measured 3 times during the EDIC study, at baseline and at years 6 and 12 [157]. 1,229 individuals from the DCCT/EDIC cohort were followed-up regarding cIMT measurements. After 6 years, the cIMT was significantly greater among study subjects compared to age- and sex-matched non-diabetics; also the progression in cIMT was greater for those with conventional insulin treatment compared to the group receiving intensive insulin treatment during DCCT. cIMT progression also positively correlated with age, sBP levels, smoking, LDL/HDL-cholesterol ratio and urinary albumin excretion rate, all known risk factors for CVD [158]. On the other hand, the 12-year follow-up analysis showed no difference in cIMT progression between the two DCCT groups from years 6 to 12 [157].

The DCCT/EDIC 13-year follow-up study revealed a lower number of macrovascular complications in the intensified insulin treatment group [101]. The relative risk reduction in mortality and morbidity CVD was 57 % [101]. It was also demonstrated that a 10 % reduction in levels of HbA_{1c}, during the DCCT, was accompanied by a 20 % decreased relative risk of subsequent CVD events [101]. These salutary effects on macrovascular complications in the intensively insulin-treated group were suggested not to be driven by differences in levels of HbA_{1c}, blood lipids, blood pressure or pharmacological treatment (although more patients in the conventional group had beta blockers). Authors speculated that the earlier 6.5 years of intensified insulin treatment, which substantially decreased HbA_{1c} levels, contributed to these long-term effects [101]. Now, nearly 30 years after DCCT commenced, 1,429 of the original study subjects have been followed up, demonstrating a modest, although statistically significant, difference in all-cause mortality rate in favor of those previously randomized to initial intensive insulin replacement therapy compared with conventional glycemic treatment [22].

2.8 STOCKHOLM DIABETES INTERVENTION STUDY (SDIS)

The DCCT and SDIS studies both demonstrated almost identical results, *i.e.* that early intensive insulin treatment can postpone or prevent microvascular complications in type 1 diabetes patients [45, 96]. The treatment effects were, by any standard, very impressive.

The SDIS was initiated by the late Dr. Per Reichard in 1982 in order to investigate whether intensive insulin therapy could reduce the incidence of microvascular complications in type 1 diabetes patients in Sweden [45]. One hundred and two patients were randomly assigned to receive either intensified conventional treatment (ICT) or standard treatment (ST) for a period of 7.5 years. Inclusion criteria were type 1 diabetes (diagnosed before the age of 31, and requiring insulin treatment within one year), no or non-proliferative retinopathy, and normal urinary albumin excretion rate and glomerular filtration rate. Exclusion criteria were pro-

liferative or photo-coagulated retinopathy and known drug or alcohol abuse. The treatment regimen in the ICT group consisted of four daily injections of insulin while the majority in the ST group received two daily injections of insulin. Patients were evaluated regarding microvascular complications after approximately 18 months [159], 3 and 5 years of study start [160][161], and at the end of the study [45]. A 10-year follow-up was also made with respect to mortality and treatment side effects [45, 159-162].

The results were strikingly similar with the DCCT findings (published a few weeks later), with a significantly lower mean level of HbA_{1c} 54 mmol/mol (DCCT 7.1%) in the intensive treatment group, as compared to 70 mmol/mol (DCCT 8.5%) in the conventional treatment group, and a corresponding reduced risk of severe retinopathy, nephropathy and neuropathy in the intensified insulin treated group. During the SDIS, four patients died in the intensive treatment group and three in the conventional treatment group and -- as in the DCCT -- a three-fold higher incidence of serious hypoglycemia was noted in the intensive treatment group, and a borderline significant weight gain [163].

In the 10-year follow-up of SDIS, the glycemic control was still significantly better in the intensive treatment group despite the fact that the randomization had ceased and treatments were individualized. However, even at this late time point, the microvascular complications were even more prevalent in the conventionally treated group [45, 159-162].

Although the number of participants in SDIS was relatively few and the study primarily was designed (and statistically powered) to study microvascular complications, some sub-studies (after 7.5 years) were nonetheless performed to evaluate any differences in macrovascular complications between the two groups. Fifty nine of the subjects were tested for risk markers of CVD such as flow mediated vasodilatation in the brachial artery (an established surrogate marker for endothelial dysfunction), cIMT, arterial wall stiffness calculated and the carotid arteries scanned for plaques [164]. The results showed a better outcome for the previously intensively treated group with thinner cIMT and less stiff arteries. Moreover, among all participants, patients with lower levels of HbA_{1c} had less stiff arteries and a better endothelial function [164].

2.9 GLYCEMIC MEMORY

Although the mean HbA_{1c} converged between the two treatment groups in DCCT soon after study end, the effect of the difference treatment intensity during the trial continued to cause more complications in the previously conventionally treated group [101, 155, 156]. This phenomenon was later on described as a glycemic memory. The same phenomenon was observed in the UK Prospective Diabetes Study (UKPDS), studying newly diagnosed type 2 diabetes individuals [149]. In the DCCT/EDIC trial, this glycemic memory effect has remained over the years, as was recently demonstrated for both microvascular [165, 166] and macrovascular complications [22, 167].

One suggested mechanism behind the purported glycemic memory is epigenetic alterations, *i.e.* non-hereditary modifications gene expression efficacy by for instance methylation without affecting the underlying genomic sequence [168]. Epigenetic modifications can also be caused by alterations in the environment [169] and by exercise. Experimental models have demonstrated that a high-glucose environment causes alterations in epigenetic post-

translational histone modification affecting inflammation and vascular complications [170, 171]. Indeed, it was recently demonstrated that epigenetic histone modification is involved in the glycemic memory in study subjects from the DCCT/EDIC cohort trial [172].

2.10 REVASCULARIZATION IN TYPE 1 DIABETES

Established CHD is a strong risk factor for recurrent events, especially if combined with diabetes [173]. Coronary artery bypass grafting (CABG) is, together with percutaneous coronary intervention (PCI), currently the methods of choice for interventional coronary revascularization therapy in CHD. Patients with known or previously undiagnosed diabetes constitute a large proportion of the CHD population, up to 25 % [174]. This figure is even further increased if subjects with pre-diabetes (*i.e.* impaired glucose tolerance) are included. Revascularization surgery for patients with multivessel CHD is a common procedure. Patients with diabetes and established CHD often have more disseminated and aggressive atherosclerosis and are at much higher risk of developing major adverse CVD events and death than those without diabetes [175-178].

The outcomes of most studies have favored CABG over PCI as the preferred type of intervention, not least in diabetic subjects since they (as mentioned above) often have multi-vessel disease [179]. A recent meta-analysis, based on eight studies including some 300,000 subjects, also found outcome in favor of CABG compared to PCI [180]. In the Bypass Angioplasty Revascularization Investigation (BARI) trial, a randomized controlled trial comparing CABG and PCI was conducted and patients were followed for 5.4 years. As compared to CABG, an initial choice of PCI did not significantly compromise five-year survival [181]. However, in a sub-analysis of diabetic patients (not specified by the protocol), the five-year survival rate was significantly increased in patients randomized to CABG [181].

In the Strategies For Multivessel Revascularization in Patients with Diabetes (FREEDOM) trial, the hypothesis of CABG being more favorable than the introduction of PCI with drug-eluting stents among diabetes patients for long-term outcome was tested [182]. The five year event rates of all-cause mortality, non-fatal myocardial infarction and non-fatal stroke were all significantly lower in the CABG group compared to PCI [182]. The stroke frequency, on the other hand, was higher in the CABG group, being consistent with many other studies and demonstrated in a recent meta-analysis of 19 RCTs with 10,944 subjects [183]. The higher rate of stroke has been suggested to be caused by more extensive coronary artery disease, perhaps being typical for diabetes patients.

3 HYPOTHESIS

In this thesis, the following hypothesises were tested. First (**Study I**), we hypothesized that young type 1 diabetes individuals have more signs of atherosclerotic development, measured by means of cIMT as a surrogate marker, and that they are more insulin resistant compared to subjects of a matched healthy control group. Second (**Study II-III**), despite the fact that SDIS was not statistically powered to address macrovascular complications, we hypothesized that the unusually long duration of this study may compensate for the relatively small number of study subjects, and that differences in macrovascular outcomes between groups might therefore be possible to detect. Moreover, we also hypothesized that glycemic memory, or rather the earlier intensive insulin therapy, may explain the putative differences in outcomes between those groups. Since numerous studies have demonstrated a shorter longevity and an increased risk of CVD related mortality in type 1 diabetes, as compared to non-diabetic subjects, we hypothesized (**Study IV**) an increased risk of death in type 1 diabetes individuals, *vs*. non-diabetic subjects, after CABG.

4 AIMS

Briefly, these projects focus on macrovascular complications in individuals with type 1 diabetes in different settings. It started out with the aim to investigate early signs of atherosclerotic lesions in adolescent and young adult type 1 diabetes patients. Thereafter, the individuals, previously randomized to receive either intensive insulin replacement therapy or standard treatment in the SDIS cohort were studied with regard to all-cause mortality, mortality/morbidity in CVD, diabetic nephropathy, and ischemic foot ulcer. The last project was aiming at exploring the outcome of macrovascular complications in type 1 diabetes patients undergoing CABG and to compare the incidence of such complications with type 2 diabetes patients and non-diabetics.

The specific aims of this work were:

- To investigate early signs of atherosclerotic lesions, measured by using cIMT as a surrogate marker, and their relation to insulin sensitivity in young type 1 diabetes individuals.
- To compare skin microvascular function in the foot and time to first hospitalization for ischemic foot ulcer in patients from the two groups in the SDIS.
- To compare the incidence of all-cause mortality between patients from the two SDIS cohorts. The secondary aim was to compare these two groups in terms of incidence in CVD events, *i.e.* myocardial infarction and stroke, and in diabetic nephropathy.
- To compare long-term survival in patients with either type 1 diabetes or type 2 diabetes with that of non-diabetic subjects following CABG.

5 METHODS

5.1 STUDY PATIENTS

5.1.1 Study I

In this study, 20 adolescent and young adult type 1 diabetes patients were recruited from the diabetes outpatient clinic at Sachs' Children and Adolescent Hospital. Twenty healthy peers (aged 14-20 years) were also invited by means of visiting schools in the same area as our patients and constituted a matched control group. Inclusion criteria (diabetes group) were: known type 1 diabetes, diabetes duration > 1 year, and age 14–20 years.

5.1.2 Study II and III

In previously published papers, 112 patients from four diabetes clinics in the greater metropolitan Stockholm area were initially invited to participate in SDIS. One hundred and two patients (91 %) met the inclusion criteria and were enrolled from September 1982 to March 1984. The participants were followed for 7.5 years and thereafter the randomization ceased.

In 1995, all the remaining SDIS participants (*n*=96) were invited to an investigation of skin microcirculation by means of iontophoresis. Seventy two patients (ICT=35 *vs.* ST=37) agreed and were enrolled. These 72 individuals constitute the study population in Study II (Figure 1).

The study population in study III consists of the 102 participants originally recruited to the SDIS in the early 1980´s (Figure 2).

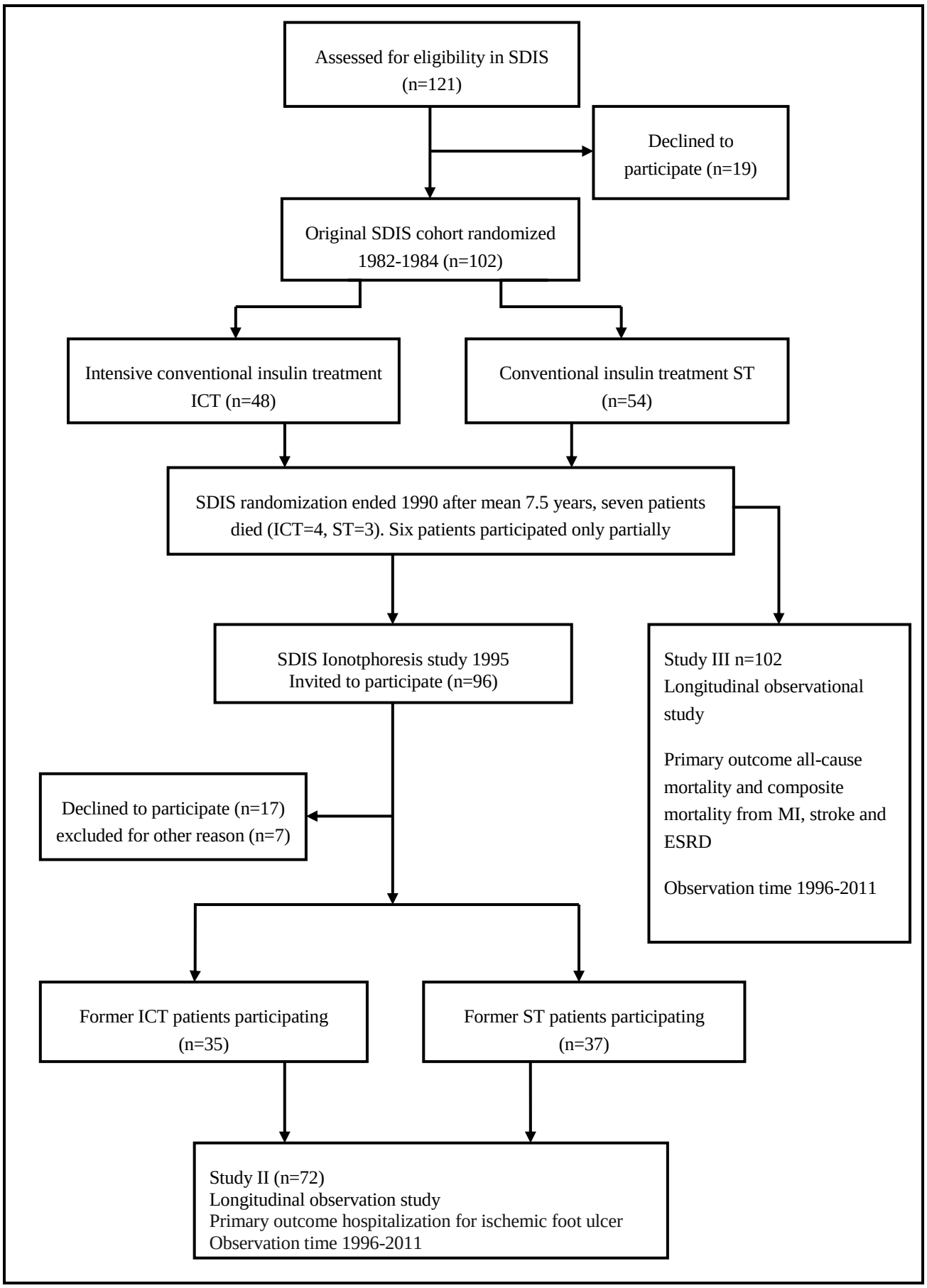

Figure 2 Flow chart outlining the original Stockholm Diabetes Intervention Study, the timing of iontophoresis, study II and III.

5.1.3 Study IV

The study population consisted of all persons, in total 39,235 patients, who underwent primary isolated non-emergency CABG in Sweden between 2003 and 2013. Patients were obtained from the Swedish Web System for Enhancement and Development of Evidence-based Care in Heart disease Evaluated According to Recommended Therapies (SWEDEHEART) register.

5.2 STUDY PROTOCOLS

5.2.1 Study I

This was a cross-sectional study. After a 12 hours overnight fast, blood test were drawn for analyses of blood lipids, creatinine and HbA_{1c}. BMI, waist circumference, and blood pressure were also measured. Thereafter a hyperinsulinemic euglycemic clamp was performed to measure insulin sensitivity. After the clamp procedure, cIMT was measured with high-resolution ultrasonography.

5.2.2 Study II and III

Patients in the ICT group received extra education, including how to use self blood glucose tests several times daily. They had continuous tutoring by telephone and scheduled visits to the diabetology outpatient clinic every 2nd month. The ST patients were instructed how to use self blood glucose tests. They received no extra education and only discussed the treatment at regular visits to the outpatient clinic every four months. Glycemic control was measured by means of HbA_{1c} levels every four months for all participants. The treatment regimen in the ICT group consisted of four daily injections of insulin, usually a pre-meal fast acting human insulin (Actrapid®) combined with long acting (Monotard®) insulin. The majority in the ST group received two daily injections of insulin, usually a mixed insulin (Mixtard 30/70®). The glycemic goal was to improve glycemia to achieve an HbA_{1c} level < 52 mmol/mol (DCCT: 6%). Seven and a half years after the first inclusion into the SDIS, the randomization ceased and both the ICT and ST patients continued visiting the attending diabetologist according to the standard diabetes care at the time. The ST patients were now given advice on how to further improve their insulin replacement therapy in order to improve their HbA_{1c} levels. Ten years after the randomization, 71 % of ST group and 89 % of the ICT group were treated with at least three daily insulin injections [162].

Study II was a prospective follow-up study (Figure 2). Seventy two patients from the original SDIS cohort were evaluated regarding skin microcirculation with iontophoresis topically applied with the following vasoactive stimuli: acetylcholine (ACh) [endothelial-dependent vasodilatation], sodium nitroprusside (SNP) [endothelial-independent vasodilatation], and capsaicin [C-nociceptive-dependent vasodilatation]. HbA_{1c} levels were prospectively collected from 1990-1995 and tested for association with skin microcirculation. The cohort was then followed up as described in study III until the 31st of December 2011 and primary outcome was defined as the first recorded hospitalization for ischemic foot ulcer.

Study III was a longitudinal and observational study (Figure 2). Primary outcome was all-cause mortality, mortality in stroke, myocardial infarction and kidney failure and secondary outcomes were morbidity in myocardial infarction, stroke and ESRD. For the period January

1996 to December 2011, outcome data were extracted from the Swedish Death Register and the Swedish National Inpatient Register (IPR). Patients' medical records were obtained from Melior® and Take Care® as well as data from the Swedish national registry of diabetes (NDR). HbA_{1c}, lipid profile, blood pressure, microalbuminuria, smoking habits and use of acetyl salicylic medications, antihypertensive and lipid lowering agents were collected and the latest available data (2011) was recorded. Mean HbA_{1c} levels were also analyzed for the entire period from 1996-2011.

5.2.3 Study IV

Study IV was an observational, nation-wide population-based cohort study. Baseline characteristics were obtained from the SWEDEHEART register, and further expanded by cross-linking the data with information from the Swedish Cause of Death Register (CDR), the National Inpatient Register (IPR), the NDR, the Swedish Renal Register, and socioeconomic data from Statistics Sweden at the Swedish National Board of Health and Welfare (Figure 3). The type of diabetes was obtained from NDR. The definition of type 1 diabetes was onset of diabetes before the age of 30 years and treatment with insulin only. Type 2 diabetes was defined as diabetes treated with diet or oral hypoglycemic agents alone, or age 40 years or older at onset and treated with insulin alone or in combination with oral hypoglycemic agents. All persons not included in the NDR were defined as non-diabetic. The data were censored at the time of death or at the end of the follow-up period (March 24, 2014), whichever occurred first.

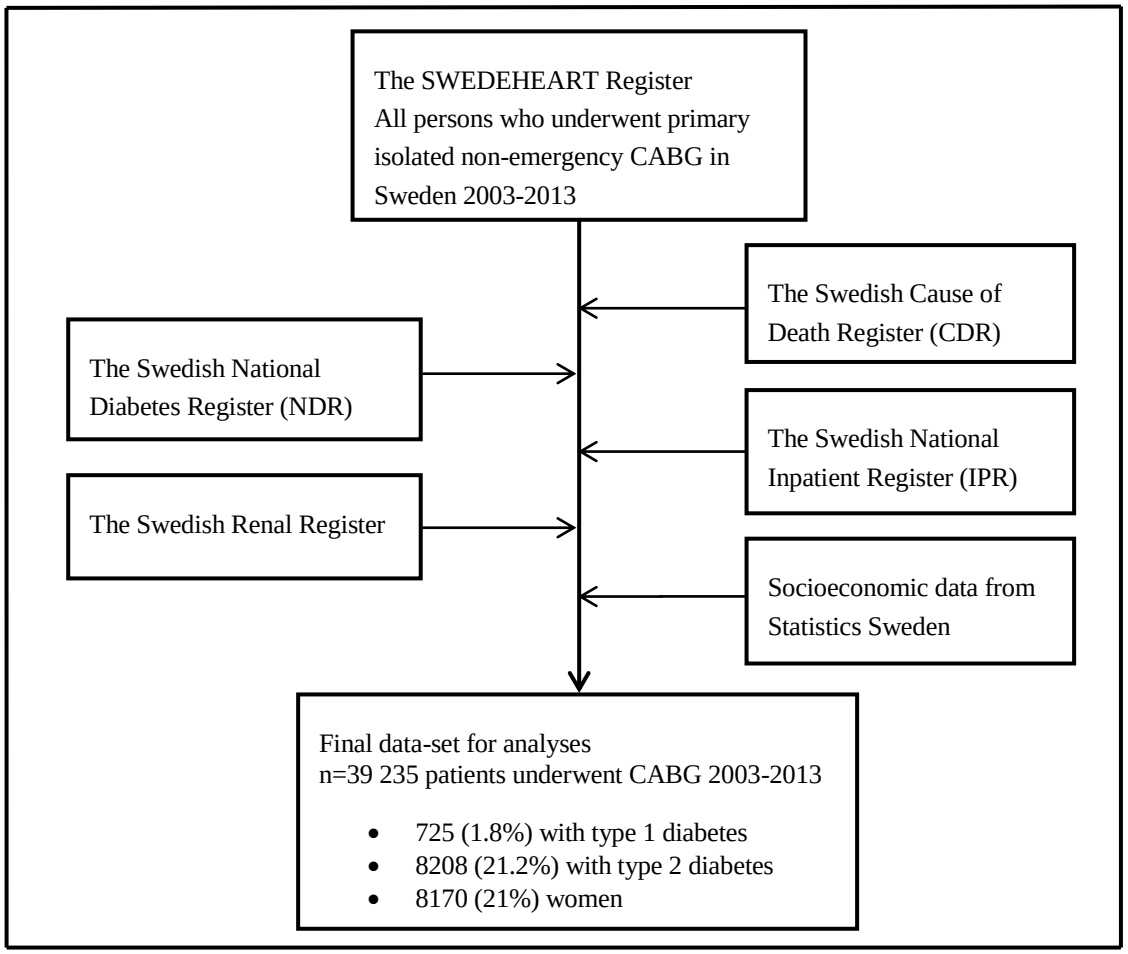

Figure 3 Flow Chart comprising data collection in study IV

5.3 STUDY OUTCOME

5.3.1 Study I

Primary endpoint was defined as mean group difference in cIMT and its association to insulin sensitivity (measured by glucose infusion rate) obtained from the clamp. Secondary endpoints were to explore the association between BMI, waist circumference, sBP, triglycerides, HDL-cholesterol, HbA_{1c} on one hand, and the measurements of cIMT and cross-sectional intima-media area (CIMA), on the other hand.

5.3.2 Study II

Primary outcome was the time to first hospitalization for ischemic foot ulcer, defined by ICD discharge codes in the IPR and confirmed by adjucating medical records with reference to the diagnostic criteria for ischemic foot ulcer, defined as ongoing foot ulcer together with ankle–brachial index <0.9 and/or toe pressure <50 mmHg. Secondary outcomes were to explore changes in skin microvascular circulation, measured by laser Doppler flowmetry, after topical administration of ACh, SNP and capsaicin, by means of iontophoresis technique and its association to which group study participants were randomized to in the original SDIS study.

5.3.3 Study III

The primary outcome was all-cause mortality and a composite mortality from myocardial infarction, stroke and ESRD. The secondary outcomes were morbidity (disease-free survival) from CVD complications, *i.e.* myocardial infarction and stroke, or ESRD.

5.3.4 Study IV

The primary outcome was all-cause mortality. Secondary outcome measures were CVD death and any major adverse coronary event (MACE), defined as a combination of hospital stay for myocardial infarction, heart failure, stroke or need of revascularization, either by PCI or CABG.

5.4 ROUTINE BIOCHEMICAL ANALYSES

In study I, the local clinical chemistry laboratory was used for analyses of HbA_{1c}, blood lipids, hsCRP, and plasma/urine creatinine levels. All the tests were drawn in the morning before the clamp. Blood glucose levels taken during the clamp were determined by the glucose oxidase method with a glucose analyzer (2300 STAT PLUS; Yellow Springs Instruments, Yellow Springs, OH, USA). Serum insulin and C-peptide levels were analyzed locally by an immunometric method with monoclonal antibodies (Modular E 170, Roche Diagnostics Scandinavia, Stockholm, Sweden).

In SDIS, HbA_{1c} levels were measured in both groups at study entry, after 6 months and then approximately at every 4 months. Mean HbA_{1c} levels were calculated for the whole 7.5 year period in the original study, as well as between 1990 and 1995 in the 10-year follow-up. In our follow-up period from 1996-2011 (study II-III), analyses of HbA_{1c} and total cholesterol levels were performed by the local clinical chemistry laboratory whenever each participant had their routine visits.

5.5 HYPERINSULINEMIC CLAMP

The hyperinsulinemic euglycemic clamp is considered to be the gold standard method for determining insulin sensitivity [184]. The aim of the clamp is to increase the plasma insulin concentration level and maintain this until steady-state (usually 120 minutes). To avoid hypoglycemia, and to keep the blood glucose concentration at a constant level, a variable glucose infusion is given. The high plasma insulin concentration is estimated to cause a halt in liver glucose production so that in the "steady-state" condition endogenous glucose production is suppressed and the glucose infusion rate during steady-state equals the net tissue glucose utilization which mirrors the tissue insulin sensitivity (Figure 4).

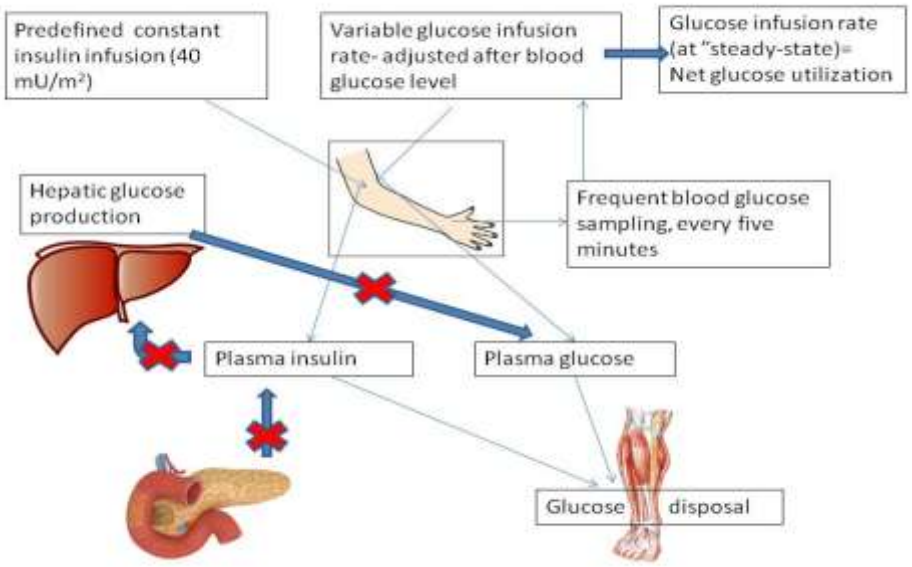

Figure 4 Outline of the hyperinsulinemic euglycemic clamp

In summary, one intravenous needle was inserted into the antecubital vein on the left arm and a second one, in a retrograde fashion, on the back of the right hand. The hand was kept warm with an electric device for intermittent sampling of arterialized venous blood. In the left arm needle, human Actrapid insulin (40 mU/m^2 NovoNordisk A/S, Copenhagen, Denmark) was infused along with 20 % dextrose (Fresenius Kabi, Stockholm, Sweden). Blood glucose samples were taken from the right hand vein catheter every 5 minutes and the rate of dextrose infusion was adjusted to achieve a blood glucose level of 5.0 mmol/l through the 120 minute clamp. Whole-body insulin sensitivity (S_i) was measured as glucose infusion rate (GIR), calculated from the amount of dextrose infused during the last 30 min of the clamp and expressed as mg/kg/min. The glucose clamp-derived index of S_i [S_i index; in (10^{-4} dl/kg/min)/(μU/ml)] was calculated from the GIR, corrected for body weight, during the final 30 min as follows: S_i index = GIRss/Gss x ΔIss, where GIRss is the steady state GIR (mg/min), Gss is the steady state blood glucose concentration (mg/dl), and Iss is the difference between basal and steady state plasma insulin concentrations (μU/ml). This calculation is assumed to correct for differences in prevailing, individual glucose and insulin concentrations.

5.6 CAROTID INTIMA-MEDIA THICKNESS

Measurement of cIMT is a well-accepted method used in estimating atherosclerotic changes in large vessel walls [185]. It has been used in both adult, childhood and adolescent populations [186] and to assess impact from various chronic illnesses [185][187].

In our study, the left and right carotids were examined, by one operator, using a Siemens Acuson Sequoia™ 512 Ultrasound System (Mountain View, CA, USA) with an 8 MHz linear array transducer. The subjects head was tilted in order to get the common carotid artery (CCA) just proximal to the bulb placed horizontally across the screen. Magnified pictures were frozen incidentally with the R wave on the electrocardiogram. The cIMT was defined as the distance between the leading edge of the lumen-intima echo and the leading edge of the media-adventitia echo in the far wall (Figure 4). Lumen diameter was defined as the distance between the leading edge of the intima-lumen echo of the near wall and the leading edge of the lumen-intima echo of the far wall. The distal part of the CCA, 5–10 mm proximal to the carotid bulb, was used for measurements of cIMT and lumen diameter (Figure 5). The computer system calculated the average intima-media thickness and lumen diameter of the analyzed section. CIMA was calculated using the formula $[(\text{lumen diameter} + 2 \times \text{IMT})/2]^2 \times 3.14 - (\text{lumen diameter}/2)^2 \times 3.14]$. All measurements were performed by one operator, blinded to all other data, using an automated computerized analyzing system. The computer system calculated the average cIMT and lumen diameter of the analyzed section. As cIMT of multiple measurements is most widely used, it was chosen as our primary end point measurement.

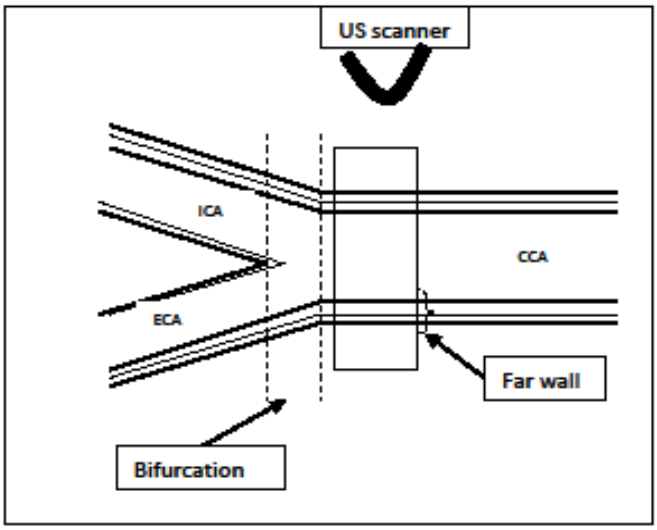

Figure 5 cIMT measurement of the common carotid artery intima media layer, area indicated by square.CCA= common carotid artery; ICA= internal carotid artery; ECA= external carotid artery

5.7 HbA_{1C}

Glucose in the blood binds irreversibly to the hemoglobin in the erythrocytes, thus creating glycated hemoglobin, *i.e.* HbA_{1c}. HbA_{1c} remains glycated during the erythrocyte's whole life span and therefore reflects average glycemia during the past six to eight weeks prior to the

sample time [188]. HbA_{1c} is firmly established as a tool for evaluating both clinical interventions and scientific investigations.

During the eighties and nineties, different countries had different HbA_{1c} standardization programs, *i.e.* the National Glycohemoglobin Standardization Program (NGSP) in the U.S., using the DCCT HPLC method as reference method and the Mono S ion exchange chromatography designated as the comparison method in Sweden [189]. Generally, the Mono S method readouts are 1.1 % (in absolute differences) lower than the DCCT method readouts. IFCC initiated a standardization of HbA_{1c}, leading to an agreement to use the units of mmol/mol as an international standard [189]. Hence, this method of measuring HbA_{1c} is used by the laboratories in Sweden since 2011. Still, the mmol/mol and DCCT % HbA_{1c} units are applied parallel in reports and medical journals and in the ADA recommendations 2015 only the DCCT % is used [7]. To convert HbA_{1c} results from NGSP (% HbA_{1c}) and IFCC (mmol/mol), the equation (NGSP = [0.09148 x IFCC] + 2.152) can be used.

HbA_{1c} analyses have been done with a HPLC method which is considered to be a "gold standard" technology. During the SDIS, the HPLC method switched to a Mono S HPLC as a reference method for measuring HbA_{1c} [190].

In study II–III, HbA_{1c} measurements for most of the study participants were analyzed at Karolinska University Laboratory. During the time of our follow-up (1996-2011), IFCC have introduced a change in reference method for calibration of HbA_{1c} [191].

5.8 eGFR

To estimate glomerular filtration rate, as a measure of kidney function, eGFR based on measurements of creatinine levels is a valid and widely used method. Contemporary calculation for eGFR is (eGFR = 170 x $[Pcr]^{-0.999}$ x $[Age]^{-0.176}$ x [0.762 if patient is female] x [1.180 if patient is black] x [serum urea nitrogen concentration SUN (mg/dL)]$^{-0.170}$ x $[Alb]^{+0.318}$). This equation is based on data from the Modification of Diet in Renal Disease (MDRD) Study [192]. Briefly, there are five stages defining chronic kidney disease based on eGFR; stage 1 (> 90), 2 (60-89), 3 (30-59), 4 (15-29) and 5 (< 15 ml/min/1.73 m^2), representing normal, mildly reduced, moderately reduced, severely reduced kidney function and very severely reduced/end-stage renal disease, respectively (from the 2012 update of the Kidney Disease Outcomes Quality Initiative, Clinical Practice Guideline for Diabetes and Chronic Kidney Disease) [193].

5.9 IONTOPHORESIS AND ANKLE-BRACHIAL INDEX

Iontophoresis is an established method by which a small applied electrical field is used to facilitate the rate of penetration of drugs into accessible tissues, such as skin (Figure 6). In combination with laser Doppler flowmetry, the technique enables access to the microvascular bed and, by applying vasoactive substances, makes measurements of changes in cutaneous blood flow amenable.

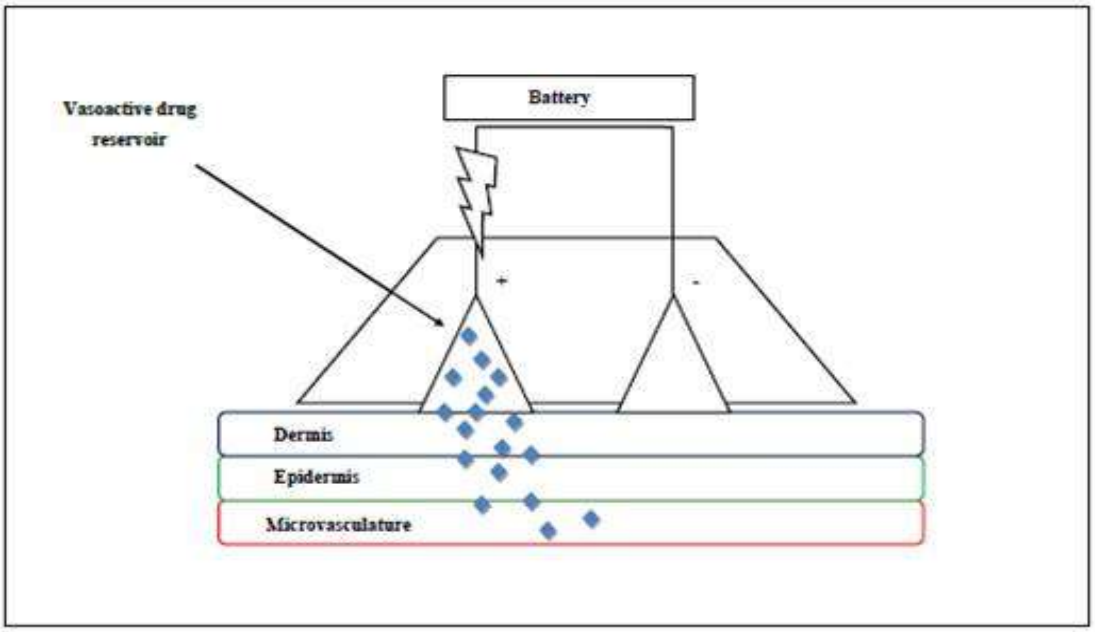

Figure 6 Principle of Iontophoresis system

A probe holder for combined single-point iontophoresis and blood flow measurement was fixed on the dorsum of the right foot. Skin blood flow at a depth of approximately 1 mm was registered by laser Doppler flowmetry technique (Periflux, Perimed, Stockholm, Sweden). Basal flow was calculated as an average of flow for 10 seconds during the last minute before the start of iontophoresis. Blood flow after administration of drugs was defined as the average blood flow for the 10 seconds with the maximal flow following the stimulation. Results are given as the change in blood flow for each of the vasoactive substances used, calculated as a ratio between the maximum blood flow after stimulation and basal flow, and presented as arbitrary perfusion units (PU). The chamber holder was filled with 2 % (wt/vol) ACh (Dispersa Hettlingen, Zürich, Switzerland) solution in de-ionized sterile water. Iontophoresis was performed with a 0.20 mA current for 15 s and repeated with a longer stimulation time (30 s) and maximal stimulation of 60–120 s. At a different site on the same foot, and not simultaneously, 2 % (wt/vol) SNP (Nipride, Hoffman-La Roche, Basel, Switzerland) and 2 % (wt/vol) capsaicin (Sigma-Aldrich, St. Louis, MO, USA) in solutions in de-ionized sterile water were iontophoresed at 0.5 mA to obtain a dose–response curve with the same time points as followed previously. For the ACh we used an anodal current, while for the SNP and the capsaicin we used a cathodal current. The investigator and the interpreter of the results were blinded to the former allocation of the individual patient.

5.10 NATIONAL REGISTERS

THE SWEDISH NATIONAL CAUSE OF DEATH REGISTER AND INPATIENT REGISTER

The CDR and the Swedish National IPR were initiated in 1964 by the Swedish National Board of Health and Welfare. It became mandatory for all county councils to report to the register from 1984 and attained national coverage in 1987. Today, more than 99 % of all

somatic and psychiatric hospital discharges are registered in the IPR. From 2001, the register also includes outpatient visits from specialized private and public caregivers.

The diagnostic validity for myocardial infarction, stroke and renal failure in the register has been shown to be more than 90 % provided it was the principal cause of hospitalization [194-196]. A drawback is that, as of today, primary care is not covered in the IPR. On the other hand, people with type 1 diabetes are routinely followed in special care units at the hospitals in Sweden. The IPR drop-out rate for 2007 has been estimated to less than one percent but changes in the country's hospital organization make it difficult to estimate the drop-out rate particularly in the areas concerning psychiatric and geriatric care. A quality control of the data reported to the IPR is routinely performed. The control includes ascertainment that compulsory variables, *i.e.* personal registration number (unique for all Swedish citizens), hospital, and main diagnosis are reported. The validity of all variables is tested and if the data is obviously incorrect it is corrected and is sent back to the liable unit for approval.

THE SWEDISH NATIONAL REGISTER OF DIABETES

The Swedish national register of diabetes (Nationella diabetesregistret, NDR) was initiated in 1996 by the Swedish Society for Diabetology. Today, virtually all type 1 diabetes patients are included in the register [197].

SWEDEHEART

The Swedish Web system for Enhancement and Development of Evidence-based care in Heart disease Evaluated According to Recommended Therapies (SWEDEHEART) register includes all patients who have underwent coronary angiography, percutaneous coronary intervention, or cardiac surgery or were cared for at any cardiac intensive care unit in Sweden since 1992 [198]. Agreement between information in the register and the medical records was reported to be between 93 % and 97 % [198].

THE SWEDISH RENAL REGISTER

The Swedish Renal Register is a national health data registry for patients on maintenance for renal replacement therapy [199]. Every patient with chronic renal insufficiency starting dialysis treatment or receiving a kidney transplant should be reported to the register, and all dialysis and transplant units in Sweden report to the register. Reporting of patients is virtually 100 %.

STATISTICS SWEDEN

Statistics Sweden (Statistiska Centralbyrån) provides up-to-date information in a variety of areas, *i.e.* education, household economy and living conditions and is one of Europe's leading statistical agencies [200].

5.11 STATISTICAL METHODS

5.11.1 Study I

Student's *t* test was used for comparison between study groups if parameters were normally distributed; otherwise Mann Whitney test was used. Test of normality was conducted with Kolmogorov-Smirnov and Shapiro Wilks test. McNemar's and sign tests were used for dichotomous variables. Spearman test was used for the correlation data. A stepwise multivariate regression analysis was used for further testing associations between cIMT (dependent) and group (explanatory) regarding atherosclerotic risk factors (S_i, BMI, waist circumference, sBP, triglycerides, HDL-cholesterol) and HbA_{1c}. All risk factors were included and successively excluded in a stepwise order, starting with S_i.

5.11.2 Study II and III

In study II, comparisons between treatment groups were made with the Mann–Whitney U test. Contingency tables were analyzed using the $\chi 2$ test. Associations were determined with linear univariable and multiple regression analysis. The measurements of skin microcirculation for each of the vasoactive substances used (ACh, SNP and capsaicin) were log transformed in order to achieve normality and then studied in the regression analyses for predefined factors collected at the time of the iontophoresis: age, duration of diabetes, HbA_{1c}, smoking, sBP, and severe microvascular complications (*i.e.* retinopathy, nephropathy and neuropathy). Time to first hospitalization for ischemic foot ulcer was analyzed using the Kaplan–Meier method, and differences between ICT and ST groups tested with the logrank test.

In study III, comparisons between the treatment groups were made with a two-sided unpaired Student's *t* test or Mann–Whitney's U-test, depending on distribution according to the Kolmogorov–Smirnov and Shapiro–Wilk tests. Categorical data were analyzed using the $\chi 2$-test. Time to death for all-cause mortality and mortality in myocardial infarction and stroke (composite outcome), as well as time to first hospitalization for cardiovascular events, *i.e.* myocardial infarction and stroke, or ESRD, were all analyzed using the Kaplan–Meier method and differences between ICT and ST tested with logrank test.

5.11.3 Study IV

Patient characteristics were described using frequencies and percentages for categorical variables, and means and standard deviations for continuous variables. Cox regression was used to estimate the risk of all-cause mortality or a combined end point (all-cause mortality or hospital stay for myocardial infarction, heart failure, stroke, or repeat revascularization) in patients with type 1 and type 2 diabetes in a comparison with reference patients without diabetes. Crude and multivariable adjusted hazard ratios (HRs) were calculated and co-variates used in the final multivariable model were age, sex, BMI, eGFR, ESRD, hyper-tension, dyslipidemia, PAD, prior PCI, chronic pulmonary disease, stroke, prior myocardial infarction, heart failure, atrial fibrillation, left ventricular ejection fraction, EuroSCORE (*i.e.* a method of calculating predicted operative mortality for patients undergoing cardiac surgery taking patient risk factors into consideration) [201, 202], alcohol consumption, birth region, education, marital status, off-pump CABG, number of grafts and type of graft.

6 ETHICAL CONSIDERATIONS

All four study protocols are in full agreement with the declaration of Helsinki. In study I, all participating adolescents and young adults, as well as the parents of those below18 years of age, gave written informed consent before inclusion. In study II, all participants in the iontophoresis study gave their written informed consent before inclusion. All four study protocols were approved by the local ethics committees (Reference numbers: 04-867/4, 2009/623-32, 2014/1878-32/4; 92-74, 189/95, 2012/829-31/4, 2012/1852-32, 2012/829-31/4, 2012/1852-32, 2009/1667-31/1, 2013/1382-32).

7 RESULTS

7.1 STUDY I

Seven of the type 1 diabetes individuals were treated with insulin pump and 13 with multiple daily injections, having a mean insulin dose of 0.84 and 0.94 U/kg/day, respectively. There was no difference in HbA_{1c} between those treated with insulin pump and those with insulin injections (68 *vs.*77 mmol/mol, n.s.). As expected, glucose homeostasis differed between the study groups; however, there were no significant differences in BMI, waist circumference, blood pressure or in lipid profile between groups. Mild retinopathy was found in seven diabetes individuals and moderate in one. Statistical analysis showed a significant correlation between diabetes duration and retinopathy (β=0.65, p<0.001).

The assessment of cIMT showed that the type 1 diabetes individuals had a significantly increased cIMT (0.52±0.1 *vs*. 0.47±0.1 mm, p<0.01) and CIMA (9.92±0.8 *vs*. 8.94±1.3 mm^2, p<0.01), compared to their healthy peers. There was no difference in carotid lumen diameter between the study groups. Type 1 diabetes individuals were significantly more insulin resistant, demonstrated by a significantly lower GIR (5.0±2.1 *vs*. 7.1±2.2 mg/kg/min, p<0.01) compared to the non-diabetic group. Also, the glucose clamp-derived index of insulin sensitivity (S_i index; adjusting for insulin concentration during clamp) was correspondingly lower in the diabetes group compared to the non-diabetic group (10.2±6.7 *vs*. 14.8±5.7 [(10^{-4} dl/kg/min)/(μU/ml)], p<0.05), demonstrating that different plasma levels of insulin were not accounting for differences in insulin sensitivity.

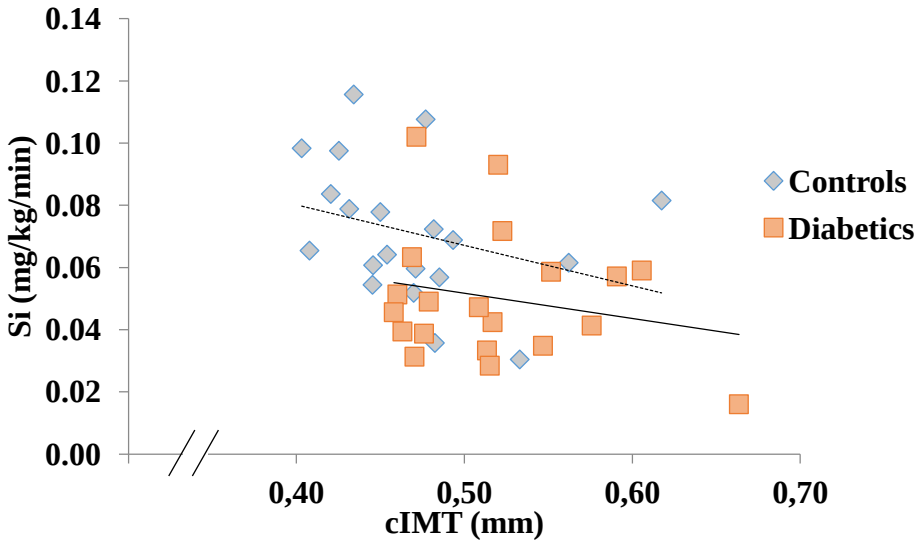

Figure 7 Association between cIMT and insulin sensitivity in diabetes individuals and controls

In the Spearman analysis for all 40 studied subjects, insulin sensitivity was negatively correlated with cIMT (r=−0.40, p<0.01), whereas waist circumference was positively correlated with cIMT (r=0.34, p=0.03). No such correlations were observed for the other risk

factors of atherosclerosis, *i.e.* BMI, sBP, triglycerides, low HDL-cholesterol levels, or for HbA_{1c}.

The association between S_i and cIMT for each group is given in Figure 7. When introducing all the atherosclerotic risk factors in a multivariate regression model, with cIMT (dependent) and group (explanatory), S_i abolished the significant association between cIMT and group, with no such effects for the other factors. Whenever the S_i factor was excluded -- but including one or more of the other risk factors in multivariate analyses -- the association between cIMT and group was, again, statistically significant. Moreover, adjustment for insulin concentration in the model did not change the results (r=0.02, p=0.07), suggesting that insulin infusion during the clamp was not a confounder.

Explanatory	**β**	**p**
Included		
All factors	0.07	<0.01
Excluded (stepwise in order)		
Si	0.08	0.11
Waist circumference	0.09	0.03
BMI	0.10	<0.01
sBP	0.09	0.01
Triglycerides	0.08	<0.01
HDL-cholesterol	0.08	<0.01
HbA_{1c}	0.05	<0.01

Table 1 Stepwise regression analysis of explanatory factors between cIMT (dependent) and group (controls and type 1 diabetes)

7.2 STUDY II

Seventy-two patients participated in the iontophoresis study, 35 from the former ICT group and 37 from the former ST group. All 72 were followed up until the end of our follow-up in 2011. Group characteristics for the ICT and ST groups revealed no statistically significant differences, except for a more frequent use of anti-hypertensive treatment in the ST group (Table 2). The incidences of nephropathy and retinopathy were significantly lower in the ICT group at the time of iontophoresis; this is in accordance with the previously published results from the SDIS [45]. The SDIS baseline levels of HbA_{1c} did not differ between study groups at the time of randomization for the entire cohort of 102 individuals (ICT 76 mmol/mol *vs*. ST 79 mmol/mol, n.s.) or *vs*. the 72 patients included in the current study (ICT 76 mmol/mol *vs*. ST 79 mmol/mol, n.s.).

Characteristic	SDIS iontophoresis (1995)		End of follow-up (2011)	
	ICT	ST	ICT	ST
n (women/men)	35 (21/14)	37 (22/15)	32 (19/13)	27 (16/11)
Age (years)	42 (28–63)	42 (31–63)	56 (45–80)	58 (49–69)
Duration of diabetes (years)	28 (19–45)	27 (19–39)	44 (36–61)	44 (37–56)
BMI (kg/m2)	24 (19–30)	24 (19–30)	25 (18–36)	25 (17–34)
Current cigarette smokers (n)	14	9	5	4
Blood pressure (mmHg)				
Systolic	135 (100–170)	135 (100–175)	130 (105–160)	135 (120–170)
Diastolic	77 (60–100)	75 (60–95)	70 (58–85)	70 (60–90)
HbA_{1c} (mmol/mol)	57 (40–79)	68** (41–96)	63 (51–90)	67 (41–98)
HbA_{1c} (%)	7.4 (5.8–9.4)	8.4** (5.9–10.9)	7.9 (6.8–10.4)	8.3 (5.9–11.1)
Total cholesterol (mmol/l)	–	–	4.5 (2.7–7.4)	5.1 (2.9–6.6)
Nephropathy (n)	2	8*	–	–
Retinopathy (n)	11	24**	–	–
Peripheral neuropathy (n)	5	10	–	–
Insulin dose (U kg body weight−1 day−1)	0.7 (0.5–0.9)	0.7 (0.4–1.0)	0.6 (0.4–1.2)	0.6 (0.3–1.0)
Anti-hypertensive medication (n)	–	–	13	25*
Lipid-lowering agent (statin) (n)	–	–	16	12
Anti-platelet therapy (n)	–	–	12	14

Table 2 Group characteristics for study II $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$

At the time of iontophoresis, levels of HbA_{1c} were lower in the ICT group, 57 mmol/mol (minimum– maximum 40–79 mmol/mol) compared with the ST group, 68 mmol/mol (41–96 mmol/mol, $p<0.01$). During the 20 year follow-up from 1996 to 2010 and in 2011, HbA_{1c} levels were similar between the groups. There were no significant differences in total cholesterol levels at any point during our follow-up period from 1996 to 2011. Cholesterol was not measured in the original SDIS study.

During the median 28 years of follow-up, 13 patients developed ischemic foot ulcer. Three patients out of 35 in the ICT group with a median follow-up of 29 years developed an ischemic foot ulcer during follow-up, compared with ten out of 37 in the ST group with a median follow-up of 28 years (logrank test p=0.035), figure 8.

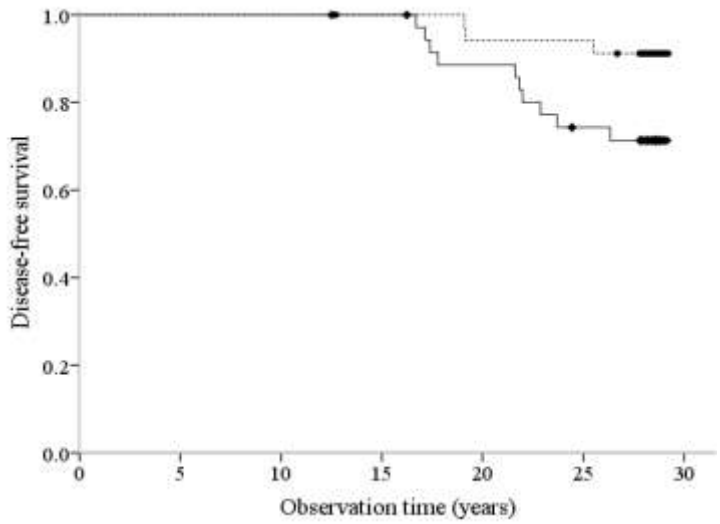

Figure 8 Ischemic foot ulcer disease-free survival. Dashed line, ICT group; Solid line, STgroup

At time of the iontophoresis, the basal skin microcirculation blood flow rates did not differ between groups (ICT 4.5 PU [1.6–13.9 PU] *vs.* ST 5.9 PU [2.1–20.0 PU], p=0.33). As expected, the healthy participants showed a higher skin microcirculation in response to all vasoactive substances tested compared with the diabetes patients regardless of former randomization. During the iontophoresis, skin microcirculation blood flow was higher in the ICT group compared with the ST group for all three vasoactive substances applied: ACh 8.1 (4.6-24.7) *vs.* 5.3 (1.7-21.4) PU, p=0.004; SNP 8.1 (2.2-20.1) *vs.* 5.6 (2.3-19.2) PU, p=0.031; capsaicin 5.0 (1.7-22.9) *vs.* 3.4 (1.5-8.4) PU, p=0.005, respectively. The differences between groups were already evident during the shortest stimulation time (15 s) when ACh or SNP were applied, and with the second shortest stimulation time (30 s) during iontophoresis with capsaicin-induced vasodilatation. ABI did not differ between study groups (ICT 1.1 PU [1.0–1.2 PU] *vs.* ST 1.1 PU [0.8–1.3 PU], p=0.54).

In the linear regression analysis against skin microcirculation results (for the maximal stimulation), a negative association was noted between mean levels of HbA_{1c} (1990–1995) and vasodilatation induced by ACh (b=−0.02, p<0.01) and capsaicin (b=−0.02, p=0.03), with a borderline association with SNP-induced vasodilatation (b=−0.01, p=0.052). There was also a negative association between severe retinopathy and ACh-induced vasodilatation (b=−0.23, p=0.047). By using the other two time points, *i.e.* 15 s and 30 s, in the linear regression analysis the results did not change. In the linear multiple regression analyses adjusting for age, duration of diabetes, smoking, sBP and severe microvascular complications, with the vasoactive substances (ACh, SNP and capsaicin) as dependent variables and HbA_{1c} as the explanatory factor, HbA_{1c} was independently associated with vasodilatation induced by ACh (b=−1.48, p<0.01) and capsaicin (b=−1.45, p<0.01), but not by SNP (b=−0.87, p=0.07).

7.3 STUDY III

At the end of the present follow-up, observational study group characteristics showed no statistically significant differences, except for a significantly higher use of anti-hypertensive treatment in the ST group (Table 3). There were no study subjects lost to follow-up, nor were there any missing data at year 2011.

Characteristics	SDIS Start (1982-1984)		SDIS End (1990)		End of follow-up (2011)	
	ICT	ST	ICT	ST	ICT	ST
n (women/men)	48 (26/22)	54 (28/26)	42 (21/21)	47 (25/22)	41 (20/21)	39 (20/19)
Age (years)	30±8	32±7	37±7	39±8	57±8	59±7
Duration of diabetes (years)	18±6	16±4	25±4	23±4	45±6	44±4
BMI (kg/m^2)	22.6±2.1	22.8±2.2	22.5±1.9	22.8±2.7	25.2±4	25.1±4
Current cigarette smokers, *n*	25	27	15	21	6	7
Blood pressure (mmHg)						
Systolic	129±14	133±15	127±13	132±15	130±15	133±15
Diastolic	77±7	79±7	78±7	78±7	70±8	72±9
HbA_{1c} (mmol/mol)	80±16	79±13	54±7	68±11	68±10	67±16
HbA_{1c} (%)	9.5±1.3	9.4±1.4	7.1±0.7	8.5±0.7***	8.4±1.0	8.3±1.4
GFR (ml min^{-1})	122±19	126±21	109±19	110±27	89±24	91±29
Insulin dose (U/kg/day)	0.73±0.21	0.75±0.22	0.73±0.19	0.71±0.21	0.66±0.24	0.62±0.25
Anti-hypertensive medication, *n*	–	–	11	17	22	33*
(ACEi or ARB-II), *n*			(10)	(14)	(20)	(31)
Lipid-lowering agent (statin), *n*	–	–	–	–	19	25
Anti-platelet therapy, *n*	–	–	–	–	29	21

Table 3 Group characteristics for study III * p<0.05, ** p<0.01 and *** p<0.001

During the median 28 years of follow-up, 22 persons died, 7 in the ICT group compared with 15 in the ST group (logrank test p = 0.15), figure 9a. Age did not differ at baseline in people who died in the ICT group 36 ± 3 years vs. the ST group 32 ± 8 years, p= 0.23. Three persons died from cancer, i.e. one male person from the ICT group died from a brain tumour and two female persons from the ST group died from a pancreatic tumour and a breast tumour, respectively. For the predefined cause-specific mortality composite endpoint, i.e. myocardial infarction, stroke and ESRD, there were no significant differences between groups, logrank test p= 0.28, figure 9b.

One person in the ICT group compared with seven in the ST group developed ESRD (logrank test p= 0.047), figure 9c. Although the chronic kidney disease stage classifications did not differ between groups, two persons (both in the ST group) out of seven who developed ESRD had a kidney replacement procedure (both persons were on dialysis and had an eGFR < 15 ml/min/1.73m^2 prior to the transplant). The duration of diabetes before ESRD in all eight persons of both groups was 37 ± 7 years. The diabetes duration for the person in the ICT group before dialysis was 33 years. All people who developed ESRD died during the follow-up. The mean survival time after the start of dialysis for all persons (both groups) was 28 ± 40 months. The primary causes of death for the ESRD people were: ESRD (two in the ST group), myocardial infarction (four in the ST group) and stroke (one person from both groups).

Eleven persons in the ICT group compared with 17 in the ST group developed myocardial infarction and/or stroke, logrank test p= 0.4, figure 9d.

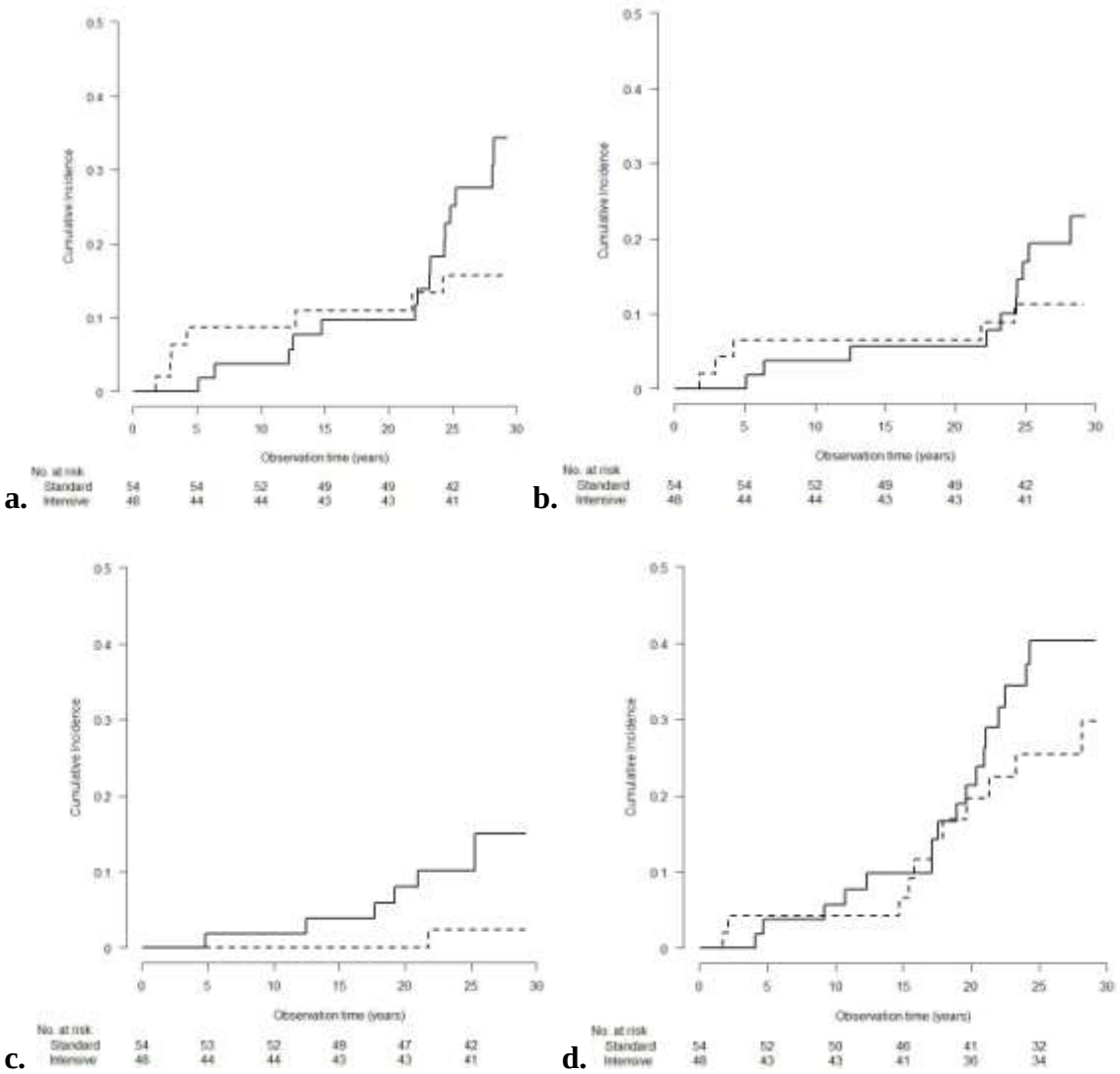

Figure 9 Kaplan–Meier curves showing Cumulative Incidence in SDIS from time of randomization until sensor date (31st December 2011) for: (**a**) all-cause mortality (logrank, p= 0.15), (**b**) composite mortality endpoint, i.e. MI, stroke and ESRD (logrank, p= 0.28), (**c**) ESRD events in (logrank p= 0.047) and (**d**) non-fatal cardiovascular events, i.e. MI and/or stroke (logrank = 0.4). Dashed line, ICT group; solid line, ST group.

7.4 STUDY IV

In total 39,235 patients with a mean age of 67 years were included, of whom 21 % (8,170) were women. There were 23 % (8,933) patients with diabetes, of whom 1.8 % (725) had type 1 diabetes and 21 % (8,208) had type 2 diabetes. Patients with type 1 diabetes were more likely to be younger, female, and have diabetes nephropathy, PAD or heart failure in comparison with patients with no diabetes or type 2 diabetes.

The mean follow-up time was 5.9 years, comprising 230,085 person-years. In total, 17 % (6,765/39,235) patients died: 17 % with no diabetes, 21 % (152) with type 1 diabetes, and 19 % (1,549) with type 2 diabetes. The age-adjusted Kaplan–Meier estimated survival curve in the 39,235 patients who underwent CABG in Sweden from 2003-2013 is shown in Figure 10.

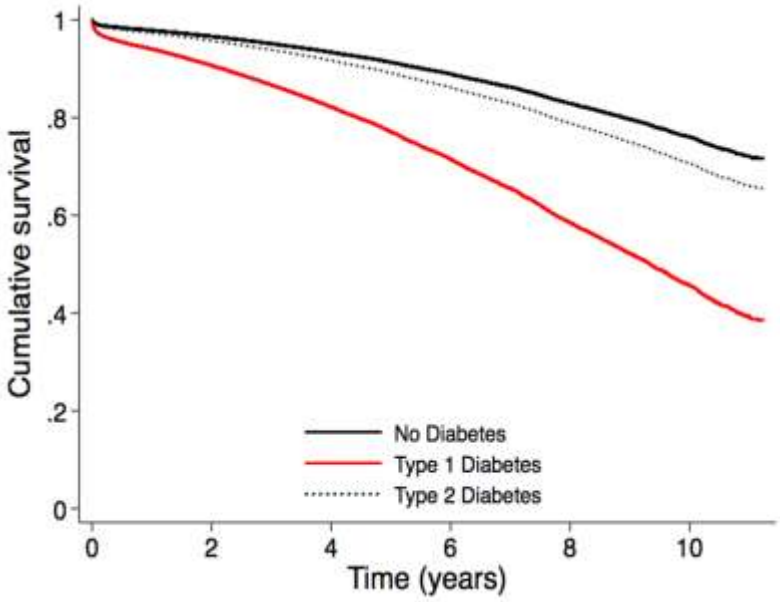

Figure 10 The crude incidence rate of death in patients with no diabetes, type 1 diabetes, and type 2 diabetes was 28 (95% CI 27–29), 39 (95% CI 33–45), and 33 (95% CI 31–35) per 1000 person-years, respectively.

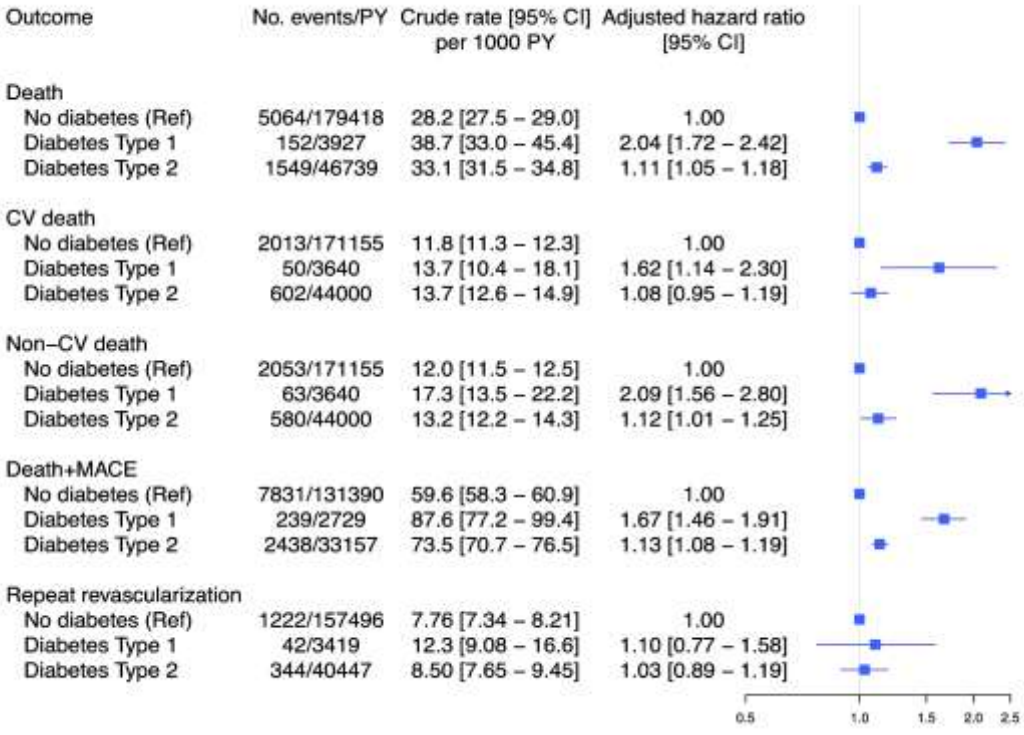

Figure 11 After 5 years of surgery survival was 89%, 85%, and 89%, respectively.

After multivariable adjustment, the HR (95 % CI) for death was 2.04 (1.72–2.42) in patients with type 1 diabetes and 1.11 (1.05–1.18) in patients with type 2 diabetes, compared with patients without diabetes. When analyzing death and MACE as a composite outcome, the

associations found were similar to those for death alone, with nearly a doubling of risk in patients with type 1 diabetes and only a small increase in risk in patients with type 2 diabetes (Figure 11). In a subset of patients with type 1 and type 2 diabetes (*n*=8,933), we found that there was a significantly higher risk for all-cause mortality in type 1 diabetes (HR 1.70 [1.40-2.06]). In 99.6 % (8,899/8,933) of these patients, information regarding preoperative HbA_{1c} level and diabetes duration was available. When HbA_{1c} levels and duration of disease were added to the multivariable model, the risk for all-cause mortality in type 1 diabetes compared with type 2 diabetes was slightly attenuated (HR 1.44 [1.14-1.80]).The patients with type 1 diabetes had a longer duration of disease (mean 40.8 *vs*. 9.6 years). The adjusted risk of death was similar among men and women with type 1 diabetes: HR 1.83 (1.45–2.30) and HR 2.17 (1.66–2.84), respectively. In type 2 diabetes, the absolute risk of death was higher in women than in men (22 % *vs*. 18 %). After adjustment for confounders, the relative risks for death and MACE were similar for both men and women with type 2 diabetes.

8 GENERAL DISCUSSION

The main findings of this thesis are that throughout life, and in different populations and concepts, type 1 diabetes renders the affected person a significantly increased risk of macrovascular complications. Adolescents and young adults with type 1 diabetes have signs of early atherosclerotic development, as reflected by increased thickening of the intima-media layer of the carotid artery walls concomitant with insulin resistance. Long term follow-up of intervention with earlier intensive insulin treatment, improving glycemic control, seems to exert a favorable impact on the outcome in future macrovascular complications, as well as for diabetes nephropathy. Also, type 1 diabetes individuals have a dire outcome after CABG interventions compared to type 2 diabetes and non-diabetes individuals in the same situation.

Insulin resistance and markers of atherosclerosis (cIMT)

There are other studies that have examined the influence of insulin sensitivity on cIMT, of which some [203-205], but not all [206], have found an association. Not only insulin resistance, but clustering of metabolic factors such as hyperglycemia, hypertension and dyslipidemia have all been demonstrated as risk factors involved in the progression of cIMT [207, 208]. In our study, levels of HbA_{1c} did not correlate with cIMT, which is in line with a previous Finnish study [209], in which diabetes duration, LDL-cholesterol and sBP all were associated to the increased cIMT [209]. In contrast, other studies demonstrate that the progression of cIMT largely is explained by differences in HbA_{1c} [157, 210], and that diabetes duration, sBP and BMI, rather could add to the risks for the increment in cIMT [211]. In the DCCT/EDIC trial, cIMT was significantly higher in all study subjects compared to a matched control group during the first six years [158]. Progression of cIMT was associated with age, blood pressure, smoking, blood lipids, microalbumiuria and levels of HbA_{1c} [158]. Also the progression of cIMT was greater for those with conventional insulin treatment, compared to the group with earlier intensive treatment during DCCT [158]. The discrepancy between our study and others, in terms of lack of association between glycemic control and cIMT, may possibly be explained by cohort size, age and diabetes duration [209, 210].

Whether cIMT is a useful marker to predict CVD is not yet established. However, the Association for European Pediatric Cardiology Working Group on Cardiovascular Prevention suggests that cIMT might be a useful tool to use in adjuvance in high risk groups [185].

Glycemic control and the progression of macrovascular disease

SDIS and DCCT clearly demonstrated that intensified insulin treatment and improved glycemic control retards microvascular complications in type 1 diabetic patients [44, 45]. The two trials share many features regarding population and study protocol, as well as the timing. Although the DCCT cohort was 15 times larger than SDIS, the primary results on macrovascular complications were borderline significant [44]. Later follow-ups in DCCT/EDIC were on the other hand undisputable in the favor of intensified insulin replacement therapy in preventing CVD events [22, 101].

We found in our SDIS follow-up study that the previously intensified insulin-treated individuals had significantly fewer ischemic foot ulcers and ESRD events. Although, all-cause mortality, composite mortality (myocardial infarction, stroke and ESRD), CVD mortality or CHD and stroke events did not differ statistically between groups, all the outcomes point in the same direction, *i.e.* with fewer cases in the previous ICT group. One can argue that these findings might purely be by chance. SDIS was a small cohort and therefore underpowered and never designed to study macrovascular outcome. On the other hand, previous reports from the SDIS cohort have demonstrated that markers of atherosclerosis can be halted by intensified insulin treatment [164].

In contrast to the DCCT/EDIC cohort, the former SDIS participants have solely been followed in a routine clinical setting. The DCCT/EDIC protocol may influence the approach to signs and symptoms so that they may be more thoroughly followed-up regarding long-term complications compared to type 1 diabetes patients in general, *e.g.* a selection bias. The concordant results between our follow-up SDIS and the DCCT/EDIC may strengthen the assumption that the DCCT/EDIC findings are representative for every day routines for type 1 diabetes patients in general. The long-term perspective of nearly three decades in SDIS also strengthens our findings. Very recently, long-term follow-up data from the DCCT/EDIC trial demonstrated an inverse correlation between earlier intensive insulin treatment and all-cause mortality [22].

Even though the rates of diabetes-related complications have substantially declined over the last decades, there is still a large burden of the disease [15] and in fact higher for type 1 diabetes individuals compared to type 2 [212]. One explanation for this might simply be owing to the duration of the disease. An average duration of 45 years, as observed in SDIS, substantially increases the risk of complications. The same goes for the findings after CABG intervention where the type 1 diabetes patients had more than 30 year longer diabetes duration compared to their type 2 diabetes counterparts. Here the type 1 diabetes individuals had twice the mortality risk compared to type 2 diabetics [213]. The former were also more likely to have diabetic nephropathy, PAD and heart failure, thus having more advanced macrovascular co-morbidity.

Glycemic control and microvascular function

The earlier ICT group in SDIS had better skin microcirculation for all three substances used in the iontophoresis procedure, compared to the ST group. Except for long-standing hyperglycemia, no other differences in risk factors believed to affect microcirculation were observed between the two groups after multiple regression analysis. Only levels of HbA_{1c} were associated with endothelial-dependent (ACh-induced) and C-nociceptive-dependent (capsaisin-induced) skin microvascular blood flow, but not with non-endothelial dependent (SNP -nduced). C-nociceptive-nerve fibers stimulate the release of vasoactive substances, e.g. bradykinin, which in turn improve the ACh-induced endothelial function [214]. Development of foot ulcers in patients with diabetes is common with serious implications, including leg amputations [215]. The majority of leg amputations are caused by PAD in combination with skin infection [67]. Still, microvascular disturbances and progressive neuropathy most often precede the development of foot ulcers [216]. Therefore, the iontophoresis results align very well with this and, as our treatment groups do not differ in risk factors other than glycemic control, it suggests that the increased long term propensity for foot ulcer development may to a large extent be caused by hyperglycemia-induced deterioration in skin microcirculation.

Glycemic control and diabetes nephropathy

We found that intensive insulin treatment for an average of 7.5 years was associated with significantly lower incidence of ESRD compared with individuals randomized to standard insulin treatment in the SDIS cohort. Since there were multiple secondary outcomes and no correction was made for multiplicity, this result should be interpreted with some caution.

It was demonstrated in the DCCT and SDIS that intensive insulin therapy halted the progression of diabetes nephropathy [45, 96]. Impairment of GFR is the unified common pathway for renal failure, *i.e.* ESRD. Diabetic nephropathy, including ESRD, clearly adversely impacts the mortality rate [24, 25], which was also observed in the SDIS follow-up study where patients had a mean survival rate of only 28 months after the start of dialysis. Undisputedly, glycemic control at today's recommended levels can protect from and halt the development of diabetic nephropathy, which in turn impacts the risk of future macrovascular complications. Interesting and promising findings from the DCCT/EDIC study indicate that sustained reversal of diabetic nephropathy is amenable and primarily seems associated to lower HbA_{1c} and antihypertensive treatment, mainly with Renin-Angiotensin inhibitors [84]. These findings are hopeful and provide impetus for continuous efforts in early improvement in glycemic control and treatment of albuminuria and hypertension [123, 124].

Adverse effects of improvement in glycemic control

There is a relationship between hypoglycemia and CVD mortality in type 1 diabetes [144]. In our studies, we did not investigate hypoglycemic events in association to macrovascular complications. However, both the DCCT and the SDIS trials recognized increased incidence of severe hypoglycemia in the intensively insulin-treated groups [44, 45]. Earlier follow-up studies from these trials have not shown any differences in cognitive function [135, 162]. In contrast to this, cross-sectional studies have demonstrated some detrimental impact of severe hypoglycemia on cognitive function in type 1 diabetes individuals [217, 218].

Obesity is a risk factor for the progression of subclinical atherosclerosis into overt macro-vascular complications. However, we did not observe any changes in weight or BMI at follow-up in the SDIS. Others have expressed concerns regarding weight gain as a result of intensified insulin treatment [134]. In the DCCT/EDIC trial, it was demonstrated that patients with the largest weight gain (both DCCT intensive and conventional treatment subjects) had a greater cIMT at both year 1 and 6 [135].

Glycemic memory

The long-term outcomes from the SDIS, may suggest a role for glucose memory, in which the 7.5 years of diverging glycemic control between treatment groups eventually vanished [164, 219]. The DCCT/EDIC and the UKPDS findings of a persistent effect of intensified anti-hypergycemic treatment on the development of macroangiopathy are more or less unequivocal today [22]. Also, an increasing number of experimental studies where a high glucose environment can cause alterations in epigenetic post-translational modifications -- for example affecting inflammation and vascular complications [170, 171] -- offer mechanistic insights into this phenomenon. Similar findings from the DCCT/EDIC cohort suggest such mechanisms to be at least part of the explanation behind the glycemic memory [172].

One main finding from the DCCT/EDIC studies has been that elevation of HbA_{1c}, both at study entry and during the randomized treatment period, is associated with the risk of progression of complications [152]. It was also recently suggested that levels of HbA_{1c}, very early from the onset of type 1 diabetes, *i.e.* already in children and adolescents, may predict the risk of developing complications later on [220], indicating a strong and early need for rigorous metabolic control.

9 CONCLUSIONS

Throughout the work with the thesis, the over-arching goal has been to describe diabetes complications as a continuum gradually developing in a life-time perspective. We have found in our studies on type 1 diabetes that:

- Adolescent and young adult type 1 diabetes individuals show early signs of atherosclerosis compared to a matched control group. The marker of atherosclerosis identified (cIMT) is associated to insulin resistance.
- Earlier intensive insulin treatment and improvement of glycemic control preserve skin microcirculation and seem to exert a favorable impact on incidence of foot ulcers in a long-term perspective.
- Earlier intensive insulin treatment also seems to have a favorable impact on incidence in diabetes nephropathy in a long-term perspective.
- Type 1 diabetes individuals have a poorer outcome after CABG intervention compared to type 2 diabetics and non-diabetics in the same situation.

10 STUDY LIMITATIONS

Study I was an explorative study with no power analysis conducted. Also, since this study was cross-sectional no conclusions can be made regarding causal relationship between cIMT and insulin resistance. The SDIS cohort was small and was not primarily designed for analyses of macrovascular complications. In study III, no correction was made for multiple comparisons. The results from the CABG follow-up study are only applicable for this intervention as no data were analyzed regarding PCI. On the other hand, CABG is today considered the treatment of choice in patients with diabetes.

11 FUTURE PERSPECTIVES

In the DCCT/EDIC trial, excessive weight gain as a result of intensified insulin treatment was a cause for concern [135]. I believe there is a need to explore treatment regimens for handling unsatisfactory glycemic control and weight gain, and to further explore the role of adjuvant type 1 diabetes treatment that traditionally has been traditionally been targeting type 2 diabetes, metformin [221], incretin-based therapy [222], and sodium glucose transporter-2 (SGLT2) inhibitors [223]. Some of these anti-diabetic agents may even have beneficial effects on the vasculature beyond glucose control [224].

In conjunction with this, the impact of insulin resistance and possible gender differences in development of long-term CVD complications in type 1 diabetes individuals would be interesting to investigate. One such example would be to investigate whether eGDR could predict CVD events in type 1 diabetes individuals.

Since type 1 diabetes individuals have an increased risk of adverse outcomes after CABG [213] , one obvious issue to address is whether preoperative treatments could predict CVD events or death after such a procedure. It would also be of great interest to investigate whether hypoglycemia or variability of the same, or variability of glycemic control may predict CVD events or death after CABG in type 1 diabetes individuals.

Recent analyses by the DCCT/EDIC group [225] confirmed the previous findings of lack of impact of severe hypoglycemia on cognition. They instead found an association between high levels of HbA_{1c} and mild cognitive dysfunction. Microvascular complications, smoking, hypertension and increased levels of HbA_{1c} are all risk factors affecting cognition and psychomotor function [226]. Microvascular complications, associated with chronic hyperglycemia, may affect cerebral microvasculature causing structural and functional impairment [227, 228]. These issues need to further be studied.

12 SVENSK SAMMANFATTNING

Risken att insjukna eller avlida i hjärt- och kärlsjukdom i förtid är betydligt större för personer med typ 1 diabetes jämfört med personer utan diabetes. Utveckling av mikroangiopati och tecken på makrovaskulära komplikationer föregår insjuknandet. Stockholm diabetes intervention study (SDIS) och, något senare, den amerikanska DCCT/EDIC studien visade redan 1993 mycket tydligt att tidig intervention med intensifierad insulinbehandling minskade de mikrovaskulära komplikationerna DCCT/EDIC studien har senare också visat motsvarande resultat med minskad risk för makrovaskulära komplikationer, vilket föreslås orsakas av ett glykemiskt minne.

Denna avhandlings syfte har varit att (**studie I**) undersöka om ungdomar och unga vuxna med typ 1 diabetes har tecken på ateroskleros, mätt som carotis intima-media tjocklek (cIMT) och hur detta relaterar till deras insulinkänslighet. Vidare har vi (**studie II**) analyserat mikrocirkulationen i huden på foten och om resultatet associerar till tiden till första inläggning på grund av ischemiskt fotsår. Vi har också studerat om utfallet skiljer sig mellan de båda tidigare studiegrupperna i SDIS som genomgick intensiv insulinbehandling eller standardbehandling med insulin under 7.5 år. Vi har också genomfört en långtidsuppföljning (**studie III**) där vi studerat incidensen i död oavsett orsak, död i hjärt- och kärlsjukdom eller terminal njursvikt liksom insjuknande i hjärt- och kärlsjukdom, dvs. hjärtinfarkt, stroke och terminal njursvik i de båda studiegrupperna i SDIS kohorten . Slutligen har vi (**studie IV**) undersökt långtidsöverlevnaden efter genomgången koronar bypass kirurgi (CABG) mellan personer med typ 1 diabetes, typ 2 diabetes och personer utan diabetes genom att samköra data från SWEDEHEART-registret och nationella diabetesregistret. Utfall var dö oavsett orsak, död i hjärt- och kärlsjukdom samt insjuknande i hjärt- och kärlsjukdom, dvs. hjärtinfarkt, hjärtsvikt, stroke och behov av revaskularisering.

I **studie I** hade ungdomarna med typ 1 diabetes signifikant ökad cIMT tillsammans med en mindre insulinkänslighet jämfört med ungdomarna utan diabetes. I en multivariat regressionsanalys var cIMT associerat till insulinkänsligheten. I **studie II** drabbades 13 patienter av ischemiska fotsår under den 28 år långa uppföljningen. Blodflödesförändringen i hudens mikrocirkulation var större hos den tidigare intensivbehandlade gruppen än hos den standardbehandlade gruppen. Blodsockerkontrollen mätt med HbA_{1c} associerade till graden av endotelberoende kärlvidgning och kapsaisin-inducerad kärlvidgning.. Under 28 års uppföljning i **studie III**, avled 22 personer. Vi fann ingen signifikant skillnad mellan studiegrupperna avseende död oavsett orsak, död i hjärt- och kärlsjukdom eller terminal njursvikt eller insjuknande i hjärt- och kärlsjukdom . En person i den intensivbehandlade gruppen och sju i standardbehandlingsgruppen insjuknade i terminal njursvikt. HbA_{1c} skilde sig inte åt mellan grupperna under de sista 16 åren av uppföljningstiden. I **studie IV** var genomsnittliga uppföljningstiden 5.9 år. Totalt avled 6765 av 39235 patienter som genomgått CABG: 17 % utan diabetes, 21 % med typ 1 diabetes och 19 % med typ 2 diabetes. Risken för död oavsett orsak var dubbelt så stor för patienter med typ 1 diabetes som för patienter med typ 2 diabetes. Risken att avlida var lika stor för män och kvinnor med typ 1 diabetes.

Ungdomar med typ 1 diabetes visar mer tidiga tecken på aterosklerosutveckling och är mindre insulinkänsliga än jämnåriga utan diabetes. Tidigare intensivbehandlade typ 1 diabetiker från SDIS studien förefaller ha en gynnsammare prognos vad gäller utvecklingen av ischemiskt fotsår och terminal njursvikt i jämförelse med de som fick standardbehandling. Detta trots att gruppernas blodsockerkontroll varit likvärdiga under de sista 16 åren av uppföljningen. Patienter med typ 1 diabetes som genomgått koronarkärlskirurgi har mycket sämre långtidsöverlevnad jämfört med personer med typ 2 diabetes.

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