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Role of alcohol and smoking for vascular complications in type 1 diabetes

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ACADEMIC DISSERTATION

To be presented, with the permission of the Medical Faculty of the University of Helsinki,
for public examination in Auditorium 2, Biomedicum Helsinki, Haartmaninkatu 8
on December 18th 2020, at 12 noon.

Helsinki 2020

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To Benjamin, Anton and Livia

"The aim of medicine is to prevent disease and prolong life,

The ideal of medicine is to eliminate the need of a physician."

William J. Mayo

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I   Harjutsalo V*, Feodoroff M*, Forsblom C, Groop P-H, FinnDiane Study Group. Patients with type 1 diabetes consuming alcoholic spirits have an increased risk of microvascular complications. *Diabetic Medicine* 31:156-164, 2014

II   Feodoroff M, Harjutsalo V, Forsblom C, Thorn L, Wadén J, Tolonen N, Lithovius R, Groop P-H, FinnDiane Study Group. Smoking and progression of diabetic nephropathy in patients with type 1 diabetes. *Acta Diabetologica* 53:525-533, 2016

III   Feodoroff M, Harjutsalo V, Forsblom C, Sandholm N, Groop P-H, FinnDiane Study Group. The impact of smoking on the effect of the rs4972593 genetic variant on end-stage renal disease. *Diabetic Medicine* 33:1301-1303, 2016

IV   Feodoroff M, Harjutsalo V, Forsblom C, Groop P-H, FinnDiane Study Group. Dose-dependent effect of smoking on risk of coronary heart disease, heart failure and stroke in individuals with type 1 diabetes. *Diabetologia* 61:2580-2589, 2018

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ABBREVIATIONS

ADA	American Diabetes Association
ADVANCE	Action in Diabetes and Vascular Disease
BMI	Body mass index
CHD	Coronary heart disease
CI	Confidence interval
CpGs	Cytosine-phosphate-guanine sites
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DCCT/EDIC	Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study
DNA	Deoxyribonucleic acid
eGDR	Estimated glucose disposal rate
eGFR	Estimated glomerular filtration rate
ER	Estrogen receptor
ESRD	End-stage renal disease
FinnDiane	Finnish Diabetic Nephropathy Study
GWAS	Genome-wide association study
HbA_{1c}	Hemoglobin A_{1c}
HDL	High-density lipoprotein
HR	Hazard ratio
ICD	International Classification of Diseases
IQR	Interquartile range
LDL	Low-density lipoprotein
OR	Odds ratio
Pittsburgh EDC	Pittsburgh Epidemiology of Diabetes Complications
SBP	Systolic blood pressure
SGLT2	Sodium/glucose co-transporter 2
SNP	Single nucleotide polymorphism
UAER	Urinary albumin excretion rate
WESDR	Wisconsin Epidemiologic Study of Diabetic Retinopathy
WHO	World Health Organization
WHR	Waist-to-hip ratio

ABSTRACT

Background

Alcohol consumption is one of the leading global health hazards that can result in premature death and disability. However, moderate alcohol consumption is associated with a decreased risk of coronary heart disease (CHD). The association between alcohol and microvascular disease entities is less studied and largely unclear. Smoking is a well-recognized risk factor for cancer, pulmonary disease, and cardiovascular disease (CVD). Previous epidemiological research has brought insight regarding the effect of both the magnitude and duration of smoking exposure on the development of vascular complications. In many countries with a high socio-demographic index, including Finland, the prevalence of smoking has decreased dramatically during the last decades, and the amount of people with a background of smoking exposure has increased. Therefore, it is relevant to explore not only the effect of active smoking but also the effect of smoking cessation on the development of vascular disease. People with type 1 diabetes are at increased risk of cardiovascular complications compared with the general population due to their metabolic disease. Therefore, it is extremely important to identify other cardiovascular risk factors in people with type 1 diabetes, to prevent further morbidity. Previous studies have linked smoking with an increased risk of micro- and macrovascular complications in people with type 1 diabetes. However, these studies have often combined current and former smokers and neglect the effect of smoking cessation. In addition, most previous studies have not included dose-dependent measures of smoking.

Aims

The aim of this thesis was to study the effect of alcohol consumption on the risk of diabetic nephropathy and severe diabetic retinopathy in people with type 1 diabetes. The effect of smoking on the development of diabetic nephropathy, CHD, and stroke was also addressed. In addition, the combined effect of smoking and a known genetic variant on the development of the end-stage renal disease (ESRD) was investigated.

Subjects and methods

All people included in the study were participants in the ongoing nationwide, multicenter Finnish Diabetic Nephropathy Study (FinnDiane), the aim of which is to identify the clinical, environmental, and genetic risk factors of micro- and macrovascular complications in people with type 1 diabetes. This thesis is based on four

studies. Study I (n=3608) is cross-sectional in nature and Study II (n=3613), Study III (n=2621), and Study IV (n=4506) are prospective. Information regarding micro- and macrovascular complications is based on data from FinnDiane visits and national data from the Finnish Care Register for Health Care and the Cause of Death Register.

Results

Compared with light consumers (<7 doses per week for men and <5 doses per week for women), people who had never consumed alcohol had a higher risk of both diabetic nephropathy and severe diabetic retinopathy. People who had given up using alcohol had the highest risk of diabetic nephropathy and retinopathy. The risk of diabetic nephropathy was increased in spirit-drinking men, and the risk of severe diabetic retinopathy was increased in all spirit drinkers compared with wine drinkers. Compared with never smokers, current smokers had a higher risk of diabetic nephropathy, both macroalbuminuria and ESRD. Former smokers had a similar risk of macroalbuminuria and ESRD compared with never smokers. In current smokers, the risk of diabetic nephropathy increased with increasing cumulative smoking, measured by pack-years. Compared with never smokers, current smokers had an increased risk of CHD, heart failure, and stroke, and former smokers had an increased risk of heart failure in the whole study population and an increased risk of stroke in men. In both current and former smokers, the risk of each cardiovascular event increased with increasing cumulative smoking measured in pack-years and increasing intensity of smoking measured in packs per day. The rare variant of allele rs4972593, previously known to increase the risk of ESRD in women, was associated with a decreased risk of ESRD in non-smoking men. In women, the increased risk of ESRD associated with the rare allele was equivalent to the risk seen in smoking women without the allele.

Conclusions

Abstaining from alcohol or previous alcohol consumption and the consumption of spirits are associated with a higher risk of diabetic nephropathy and severe retinopathy. Current smoking is associated with a higher risk of diabetic nephropathy, CHD, heart failure and stroke in a dose-dependent manner. After smoking cessation, the risk of diabetic nephropathy and CHD is decreased and approaches the risk seen in never smokers. However, the risk of heart failure and stroke remains higher in former smokers, especially in those who have smoked longer and with greater intensity. Contrary to the previous findings in women, the rare allele rs4972593 seems to have a protective effect in relation to the risk of ESRD in men.

TIIVISTELMÄ

Taustaa

Maailmanlaajuisesti alkoholi on tärkeimpiä ennenaikaisen kuoleman ja työkyvyttömyyden aiheuttajia. Kohtuullisella alkoholin käytöllä on kuitenkin todettu myös sepelvaltimotaudilta suojaava vaikutus. Alkoholinkäytön vaikutus pienten verisuonten tauteihin, kuten diabeettiseen silmänpohjatautiin ja munuaistautiin on vielä pitkälti selvittämättä. Tupakoinnin on osoitettu lisäävän riskiä sairastua erilaisiin syöpiin, keuhkosairauksiin ja sydän- ja verisuonitauteihin. Aikaisemmat epidemiologiset tutkimukset ovat osoittaneet tupakoinnin määrän ja keston vaikuttavan verisuonisairauksien kehittymiseen. Monissa kehittyneissä maissa, kuten Suomessa, tupakointi on vähentynyt huomattavasti viimeisten vuosikymmenten aikana, mikä on myös johtanut suurempaan aiemmin tupakoinnille altistuneiden henkilöiden joukkoon. Siispä onkin tärkeää tutkia aktiivisen tupakoinnin vaikutusten ohella myös aiemman tupakka-altistuksen vaikutusta verisuonisairauksien kehittymisessä. Tyypin 1 diabetes, eli insuliininpuutosdiabetes, lisää jo itsessään sydän- ja verisuonitautien riskiä. Tämän vuoksi onkin ensisijaisen tärkeää kartoittaa muita sydän- ja verisuonitautien riskitekijöitä tyypin 1 diabeetikoilla. Aiemmat tutkimukset ovat osoittaneet, että tupakointi lisää riskiä sairastua diabeteksen lisäsairauksiin. Nämä tutkimukset ovat kuitenkin usein jättäneet selvittämättä tupakoinnin lopetuksen ja tupakoinnin määrän ja keston osallisuuden diabeteksen lisäsairauksien kehittymisessä.

Tavoitteet

Väitöskirjan tavoitteena oli tarkastella alkoholinkäytön ja eri juomalaatujen vaikutusta diabeettisen silmänpohjasairauden ja munuaistaudin riskiin tyypin 1 diabeetikoilla. Lisäksi tutkittiin tupakoinnin vaikutusta diabeettisen munuaistaudin, sepelvaltimo-taudin, sydämen vajaatoiminnan ja aivotapahtuman ilmaantuvuuteen. Tavoitteena oli myös tarkastella tupakoinnin ja aiemmin löydetyn loppuvaiheen diabeettisen munuaistaudin riskiin liitetyn geenivariantin yhteisvaikutusta.

Tutkimusaineisto ja menetelmät

Tutkimus on osa FinnDiane (Finnish Diabetic Nephropathy Study) tutkimusta, joka on yhä käynnissä oleva koko Suomen kattava monikeskustutkimus, jonka päämääränä on kartoittaa kliinisiä, geneettisiä ja elintapoihin ja ympäristötekijöihin liittyviä lisäsairauksien riskitekijöitä tyypin 1 diabeetikoilla. Väitöskirja koostuu neljästä osatyöstä. Osatyö I on poikkileikkaustutkimus (n=3608) ja osatyöt II (n=3613), III

(n=2621) ja IV (n=4506) ovat luonteeltaan seurantatutkimuksia. Tiedot lisäsairauksien kehittymisestä saatiin yhdistämällä FinnDiane-aineisto valtakunnalliseen sosiaali- ja terveydenhuollon hoitoilmoitusjärjestelmään ja kuolinsyyrekisteriin.

Tulokset

Henkilöillä, jotka eivät koskaan olleet käyttäneet alkoholia, oli suurempi vaikean diabeettisen silmänpohjataudin ja munuaistaudin riski verrattuna alkoholia kohtalaisen vähän käyttäviin (miehet alle <7 ja naiset <5 annosta per viikko). Suurin silmänpohja- ja munuaistaudin riski oli henkilöillä, jotka olivat lopettaneet alkoholin käytön. Väkevien alkoholijuomien käyttöön liittyi kohonnut vaikean silmänpohjataudin riski viininjuontiin verrattuna koko tutkimusaineistossa ja miehillä myös kohonnut munuaistaudin riski. Tupakoimattomiin verrattuna nykyinen tupakointi lisäsi diabeettisen munuaistaudin riskiä, mitattuna sekä makroalbuminurialla että loppuvaiheen munuaistaudilla. Tupakoinnin lopettaneilla diabeettisen munuaistaudin etenemisen riski oli samaa tasoa kuin tupakoimattomilla. Myös suurempi tupakoinnin määrä ja altistuksen kesto askivuosina mitattuna liittyi lisääntyneeseen munuaistaudin etenemisen riskiin. Tupakointi lisäsi myös sepelvaltimotaudin, sydämen vajaatoiminnan ja aivotapahtumien riskiä, joka oli sitä suurempi, mitä suurempi oli tupakoinnin aiempi altistus (askivuodet) ja päivittäisen tupakoinnin määrä (aski/vrk). Tupakoinnin lopettaneilla havaittiin suurentunut riski sairastua sydämen vajaatoimintaan ja aivotapahtumiin. Aiemmassa tutkimuksessa löydetty loppuvaiheen munuaistaudin riskiä naisilla lisäävä geenivariantti rs4972593 vähensi loppuvaiheen munuaistautia tupakoimattomilla miehillä. Naisilla kyseinen geenivariantti aiheutti tupakointiin verrattavan kasvun loppuvaiheen munuaistaudin riskiin.

Johtopäätökset

Alkoholiabstinenssi, aiempi alkoholinkäyttö ja väkevien juomien kulutus lisää diabeettisen silmänpohjataudin ja munuaistaudin riskiä tyypin 1 diabeetikoilla. Tupakointi lisää riskiä sairastua diabeettiseen munuaistautiin, sepelvaltimotautiin, sydämen vajaatoimintaan ja aivotapahtumiin. Riski lisääntyy askivuosien ja päivittäisten savukkeiden määrän kasvaessa. Tupakoinnin lopettaneilla diabeettisen munuaistaudin ja sepelvaltimotaudin riski lähestyy tupakoimattomien riskiä, mutta sydämen vajaatoiminnan ja aivotapahtumien riski säilyy korkeampana tupakoimattomiin verrattuna. Toisin kuin naisilla rs4972593 geenivariantti vaikuttaisi suojaavan tupakoimattomia miehiä loppuvaiheen munuaistaudilta.

1 INTRODUCTION

Smoking is a major global health hazard, accounting for 7.1 million attributable deaths and 218 million disability-adjusted life years yearly (1). The four leading causes of death attributable to smoking are ischemic heart disease (1.62 million), chronic obstructive pulmonary disease (1.23 million), respiratory tract malignancies (1.19 million), and stroke (887 000). Among CVDs, the risk of non-fatal myocardial infarction, heart failure or stroke is 2–3 times higher in current smokers (2-4). During the last decades, the prevalence of smoking has declined in countries with a high socio-demographic index. Based on statistics from the Finnish Institute for Health and Welfare, during 2005–2018 the prevalence of current smokers in Finland declined from 30% to 15% in men and from 18% to 13% in women (5). Despite the decline in smoking prevalence, 8% of all vascular deaths and 8.5% of all deaths in Finland are still estimated to be attributable to smoking (6).

In people with type 1 diabetes, the majority of excess morbidity and mortality is due to micro- and macrovascular complications. The effect of smoking on vascular complications has been extensively studied in the general population and to some extent in people with type 2 diabetes. However, data regarding the effect of smoking on different vascular complications in people with type 1 diabetes is limited. In particular, more precise data—including dose-dependent measurements of smoking and data regarding the effect of smoking cessation on micro- and macrovascular complications—are lacking for people with type 1 diabetes.

Unlike the prevalence of smoking, current alcohol consumption has increased during the last decades in high socio-demographic index countries and particularly among women. Globally, 39% of men and 25% of women are current drinkers, but the difference in alcohol consumption between men and women varies between countries, and the disparity is lowest in high socio-demographic index countries (7). Based on a Finnish national survey from 2016, up to 88% of men and 85% of women in Finland are current drinkers (8).

Globally, 2.8 million deaths are attributed to alcohol consumption, and alcohol is the seventh leading risk factor for premature death and disability (7). In Finland, the number of deaths related directly to alcohol (alcohol psychosis, dependence, poisoning or, liver disease) is the highest among the Nordic countries. In 2015, 42.8 deaths per 100 000 capita were directly attributable to alcohol consumption in Finland compared with only 8.9 deaths per 100 000 capita in Sweden (9). The association between alcohol consumption and the risk of different disease entities is complex and depends on the

amount of alcohol consumed. In addition to the harmful effects, there is evidence of a beneficial effect of light-to-moderate alcohol consumption on some ischemic CVD entities and type 2 diabetes. The effect of alcohol consumption on microvascular disease entities is less studied. In particular, the effect in people with type 1 diabetes remains unclear.

Given the major health impact of cigarette smoking and alcohol consumption and the lack of data regarding the association between these behavioral risk factors and vascular complications in people with type 1 diabetes, the aim of this series of studies was to evaluate the effect of alcohol consumption and smoking on micro- and macrovascular complications in people with type 1 diabetes.

2 REVIEW OF THE LITERATURE

2.1 Diagnosis and classification of diabetes

2.1.1 Diagnostic criteria for diabetes

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia caused by defects in insulin secretion, insulin action, or both. According to the World Health Organization (WHO) criteria, diabetes is diagnosed if fasting blood glucose is higher than 7.0 mmol/l in repeated measurements, if any blood glucose value is 11.1 mmol/l or higher with symptoms of hyperglycemia, if the 2-h oral glucose-tolerance test is abnormal (≥11.1 mmol/l), or if glycated hemoglobin A_{1c} (HbA$_{1c}$) is 48 mmol/mol (6.5%) or higher (10, 11). The criteria for intermediate hyperglycemia (prediabetes) include impaired glucose tolerance and impaired fasting glucose. Impaired glucose tolerance is diagnosed when fasting plasma glucose is <7.0 mmol/l but the 2-h plasma glucose in an oral glucose tolerance test is ≥7.8 mmol/l and <11.1 mmol/l. Impaired fasting glucose is diagnosed when fasting plasma glucose is above the normal range (6.1–6.9 mmol/l), but the 2-h plasma glucose in an oral glucose tolerance test is normal (<7.8 mmol/l).

2.1.2 Classification of diabetes

According to the American Diabetes Association (ADA) position statement, diabetes is classified into four different categories: type 1, type 2, gestational, and other specific types of diabetes (12). Classification is based on the combination of patient characteristics, such as age and body mass index (BMI), the presence of hyperglycemia symptoms (polyuria and weight loss), and specific laboratory tests for autoantibodies and insulin production at the time of diagnosis.

2.1.3   Type 1 diabetes

Type 1 diabetes accounts for 5–10% of all cases of diabetes. It is an autoimmune disease often diagnosed in children and young adults but can also manifest in older age. Due to genetic susceptibility and environmental triggers, such as viral infections, childhood obesity, or dietary factors, autoantibodies are formed against the insulin-producing β-cells in the pancreas. This immunological process leads to β-cell destruction and gradual cessation of insulin production and eventually the need for lifelong insulin replacement therapy (13). Type 1 diabetes is often diagnosed due to milder symptoms caused by hyperglycemia, such as polydipsia, polyuria, and weight loss. But sometimes ketoacidosis, which requires treatment in the intensive care unit, is the first manifestation of the disease. The autoantibodies, such as glutamic acid decarboxylase, islet antigen 2, and insulin antibodies, can be detected months or even years before the diagnosis, and when hyperglycemia is detected, autoantibodies are found in 85–90% of patients (14). During the last decades, the incidence of type 1 diabetes has increased globally, probably due to an increased prevalence of childhood obesity and environmental determinants, such as improved hygiene associated with a decline in infectious diseases and changes in gut microbiota (15-18). In Finland, the incidence rate of type 1 diabetes is the highest in the world at around 55 per 100 000 person-years in children younger than 15 years. This is 50% higher compared with Sweden, where the incidence is the second highest in Europe (19-21). However, in Finland the incidence of type 1 diabetes reached a plateau between 2006 and 2011 and after that the incidence has declined especially in the youngest children, being around 40 per 100 000 person-years in children aged less than five (22).

2.1.4   Type 2 diabetes

Type 2 diabetes is the most common form of diabetes, accounting for 90–95% of all cases with diabetes. Type 2 diabetes is a metabolic disorder; instead of an absolute lack of insulin, the key components are insulin resistance and relative insulin deficiency. While type 1 diabetes is a disease of the pancreas, type 2 diabetes is associated with pathophysiological defects also in the liver, skeletal muscle, adipose tissue, kidneys, brain, and small intestine (23). Lifestyle-associated environmental risk factors play a crucial role in the development of type 2 diabetes, which is strongly associated with metabolic syndrome, obesity, and lack of physical activity. Type 2 diabetes is also highly heritable, particularly in those with age at onset of 35–60 (24). Large genome-wide

association studies (GWAS) and more recent exome sequencing studies have identified more than 400 genetic variants associated with the risk of type 2 diabetes (25, 26). Most are common variants with very small effects; therefore, combined polygenic risk scores have been generated to predict type 2 diabetes (27). Globally, over 460 million people have been diagnosed with type 2 diabetes, and in Finland the prevalence is estimated to be around 500 000 (28, 29).

2.1.5   Gestational diabetes and other specific types of diabetes

Based on the ADA criteria, diabetes is considered gestational if the diagnosis is made during the second or third trimester of pregnancy. If diabetes is diagnosed during the first trimester, it should be classified as pre-existing pregestational diabetes (most often type 2 diabetes). If gestational diabetes or prediabetes is diagnosed during pregnancy, special emphasis should be placed on changing one's lifestyle to minimize the risk of developing type 2 diabetes in the future.

The fourth category of diabetes is caused by other specific causes. These are monogenetic defects in β-cell function, including neonatal diabetes and different types of maturity-onset diabetes of the young. Different diseases affecting the exocrine pancreas can also cause diabetes, such as pancreatitis, cystic fibrosis, trauma, and pancreatic carcinoma. Some drugs, such as glucocorticoids, can also induce diabetes, particularly in people with pre-existing intermediate hyperglycemia (prediabetes) (12).

2.1.6   Novel methods for diabetes classification

Diabetes is a heterogeneous disease and despite modern diagnostic methods misclassification may still occur due to overlapping characteristics of different types of diabetes. In addition, especially for type 2 diabetes, the clinical presentation and prognosis of the disease varies between individuals. Therefore, novel cluster analyses based on clinical variables and genetic variants have been developed to stratify subclasses of diabetes (30, 31). Hopefully, this deeper knowledge of the nature of diabetes will help identify the patients who are at the highest risk of developing diabetes complications and will eventually lead to individually optimized treatment strategies (32, 33).

2.2 Macrovascular complications in type 1 diabetes

2.2.1 Cardiovascular disease

CVD comprises different diseases of the heart and the circulatory system that often have an atherosclerotic etiology. Clinically, the two major CVD disease phenotypes are CHD or coronary artery disease and stroke (ischemic or hemorrhagic). Other important CVD diagnoses are heart failure and peripheral artery disease. CVD is the leading cause of death, accounting for 31.5% of all deaths globally and 45% of all deaths in Europe (34). Globally, CVD mortality increased 14.5% from 2006 to 2016 (35). However, in most European countries the CVD mortality has decreased since 2003. In Europe, CHD accounts for 20% of all deaths, stroke accounts for 11%, and the other forms of CVD account for 14%. The prevalence of self-reported CVD is 9.2% in Europe and 11.9% in Finland (34).

2.2.1.1 Coronary heart disease

CHD is caused by atherosclerosis of the coronary arteries leading to clinical phenotypes that range from exercise-induced angina pectoris to acute myocardial infarction, depending on the severity of the disease. CHD is the leading cause of all health loss globally, measured by mortality and disability-adjusted life years (36). In Finland, the prevalence of CHD in people aged 50 and over is 14.3% in men and 7.1% in women (37). In people with type 1 diabetes, the risk of CHD is about 10 times higher compared with the general population, but the relative risk can be up to 20–30 times higher depending on age, sex, age at onset of diabetes, and the presence of diabetic kidney disease (38-40).

2.2.1.2 Heart failure

Instead of a single disease, heart failure is defined as a "clinical syndrome that results from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill or eject blood". Depending on the left ventricular systolic function, heart failure can be divided into two categories: heart failure with preserved ejection fraction and heart failure with reduced ejection fraction (41). CHD is the most common cause

of heart failure, either alone or in combination with hypertension. Other etiological causes are valvular diseases (e.g., sclerosis of the aortic valve), different cardiomyopathies, cardiac arrhythmias (e.g., atrial fibrillation), or more seldom inflammatory or infectious diseases (pericarditis or myocarditis) (42). Heart failure is uncommon in younger age groups, but the prevalence increases with increasing age. In Finland, the prevalence of heart failure is 5.3% in men and 2.5% in women aged 60–69, but in people 80 years and older, the prevalence is 23.7% in men and 27.3.% in women (37). Diabetes is associated with an increased risk of heart failure and people with type 1 diabetes have a 4-fold risk of hospitalization due to heart failure compared with the general population (43).

2.2.1.3   Stroke

According to the WHO, stroke is defined as "rapidly developed clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent other cause than vascular origin" (44). The etiology of stroke can be ischemic (cerebral infarction) or hemorrhagic (intracranial hemorrhage or subarachnoid hemorrhage). Globally, stroke is the second leading cause of death after CHD, causing 5.5 million deaths in 2016 (45). Stroke is also the second leading cause of disability, causing 116.4 million disability-adjusted life years globally. In Finland, the prevalence of stroke in people aged 50 and older is 6.6% in men and 6.1% in women (37). In people with type 1 diabetes, the risk of stroke is 5-fold higher compared with the general population (40, 46).

2.2.1.4   Peripheral artery disease

Peripheral artery disease is caused by atherosclerosis of the lower limb arteries, leading to impaired blood flow and eventually ischemic symptoms in the lower limbs. However, most affected patients are symptomless (diagnosed by ankle brachial index), and the characteristic claudication symptoms induced by exercise are rare compared with diffuse atypical leg symptoms (47). Only the most severe forms of peripheral artery disease lead to ulceration, gangrene, or even amputation. Peripheral artery disease, even asymptomatic, is associated with an approximately 3-fold increased risk of other CVD events and CVD mortality (48). In high-income countries, the prevalence of peripheral artery disease is around 6% in people aged 45–55, but up to 15–20% in

people aged 80–90 (49). In a study with a relatively young (mean age of 36) cohort of asymptomatic people with type 1 diabetes, the prevalence of peripheral artery disease was 12.8% (50). Based on a Swedish register study of type 1 diabetes, the cumulative risk of the most severe form of peripheral artery disease, lower-extremity amputation, was 11.0% in women and 20.7% in men by the age of 65 (51).

2.3   Microvascular complications in type 1 diabetes

2.3.1   Diabetic nephropathy (diabetic kidney disease)

Diabetic nephropathy or diabetic kidney disease is identified clinically by persistently increased urinary albumin excretion (albuminuria) and/or sustained reduction in kidney function measured by an estimated glomerular filtration rate (eGFR) below 60 ml/min per 1.73 m^2 (52). Albuminuria is assessed using timed overnight or 24-h urine collections or estimated based on the urinary albumin-to-creatinine ratio in spot urine samples. The different categories of albuminuria are presented in **Table 1**. The nomenclature has changed since the 2012 Kidney Disease: Improving Global Outcomes guideline on chronic kidney disease, and microalbuminuria and macroalbuminuria have been replaced by moderately and severely increased albuminuria (53). Many conditions, such as infection, fever, exercise, menstruation, hyperglycemia, and hypertension can transiently elevate the urinary albumin excretion rate (UAER). Therefore, the albuminuria level should be confirmed with two additional measurements during a 3–6-month period before a diagnosis of increased albuminuria can be made.

Table 1. Albuminuria categories in chronic kidney disease

Category	Normal to mildly increased	Moderately increased (Microalbuminuria)	Severely increased (Macroalbuminuria)
Timed 24-h urine collection	<30 mg/24 h	30–300 mg/24 h	>300 mg/24 h
Timed overnight urine collection	<20 µg/min	20–200 µg/min	>200 µg/min
Spot urine albumin-to-creatinine ratio	<3 mg/mmol	3–30 mg/mmol	>30 mg/mmol

Kidney function is measured by the glomerular filtration rate that can be estimated by calculation from the plasma creatinine concentration (54). There are five different categories of chronic kidney disease based on the progressive decrease in the GFR level (**Table 2**) (53, 55).

Table 2. GFR categories in chronic kidney disease

Category	CKD G1	CKD G2	CKD G3a	CKD G3b	CKD G4	CKD G5
Terms	Normal or high	Mildly decreased	Mildly to moderately decreased	Moderately to severely decreased	Severely decreased	Kidney failure
GFR (mL/min/1.73 m^2)	≥90	60–89	45–59	30–44	15–29	<15

CKD: chronic kidney disease

Microalbuminuria is the first sign of diabetic nephropathy and is often combined with elevated blood pressure and prevalent diabetic retinopathy. Before modern treatment options, the natural course of diabetic nephropathy was devastating, and after the onset of proteinuria (macroalbuminuria) kidney function declined in a progressive manner and mean life expectancy was less than five years (56). However, more recent studies have shown that albuminuria is a dynamic process, and in up to 40% of microalbuminuria cases UAER could regress back to the normal range (57).

Albuminuria can exist alone or in combination with a reduced eGFR level. However, in the final stage of diabetic nephropathy, ESRD, kidney function is severely impaired, and by definition dialysis treatment has been initiated or the patient has received a renal transplant. Diabetic nephropathy is the leading cause of ESRD worldwide as well as in Finland (28, 58, 59). Based on the Finnish Registry for Kidney Diseases, in 2017, active kidney replacement therapy (dialysis or kidney transplant) was initiated for 190 people with diabetes (106 with type 1 and 84 with type 2), which accounted for 35% of all new kidney replacement therapies (59).

The prevalence of diabetic nephropathy after 20 years of diabetes duration has decreased from 30% to 14% during the last decades, mostly due to improved glycemia and blood pressure care (60). Based on a recent Finnish study, the cumulative risk of ESRD was 7% after 30 years' duration of diabetes, but the relative risk was significantly lower in patients diagnosed after 1995 compared with those diagnosed before 1980 (61).

Based on ADA recommendations, screening for diabetic nephropathy should occur yearly by measuring urinary albumin and eGFR levels in all people with type 1 diabetes who have a duration of diabetes of ≥5 years (62). Optimizing blood pressure and glucose control is essential to reduce the risk of diabetic kidney disease and based on the Kidney Disease: Improving Global Outcomes guidelines treatment with angiotensin-converting-enzyme inhibitor or with an angiotensin II receptor blocker is recommended for all people with diabetes and hypertension. In people with type 2 diabetes, sodium/glucose co-transporter 2 (SGLT2) inhibitor is recommended to optimize glycemic control due to the beneficial effects on kidney function and CVD risk (63). In people with type 1 diabetes, SGLT2 inhibitors are also shown to have beneficial effects on glycemia, blood pressure, body weight, and albuminuria (64, 65).

2.3.2   Diabetic retinopathy

The diagnosis of diabetic retinopathy is based on fundus photographs, and retinal screening is recommended for people with type 1 diabetes 5 years after the onset of diabetes and every 1–2 years after that. Diabetic retinopathy is classified into four different stages based on the severity of the vascular changes in the retina—mild nonproliferative, moderate nonproliferative, severe nonproliferative, and proliferative diabetic retinopathy (66, 67). Macular edema, retinal thickening in the macular area due to the leakage from damaged capillaries, can occur at any stage of diabetic retinopathy and can lead to central vision loss if untreated (68). Diabetic retinopathy and diabetic macular edema are the leading causes of blindness among people of working age in developed countries. Based on a large pooled analysis of diabetes studies, after 20 years of diabetes duration the prevalence of proliferative diabetic retinopathy is 40.4%, and the prevalence of macular edema is 17.3% in people with type 1 diabetes (69).

The treatment of risk factors (hyperglycemia, hypertension, and hyperlipidemia) is crucial in preventing the initial changes in the retina and in delaying the progression of the early forms of retinopathy. However, there are also specific treatment options for the more severe forms of retinopathy. Laser photocoagulation has been used for decades and has been shown to reduce the risk of vision loss in patients with proliferative diabetic retinopathy and clinically significant diabetic macular edema (66). Advanced active proliferative diabetic retinopathy can also be treated by vitrectomy, a surgical procedure including incisions in the mid-part of the sclera anterior to the retina (68). Pharmacological treatment with anti-vascular endothelial growth factor agents

that prevent retinal neovascularization is recommended for central-involved macular edema and can also be used for proliferative diabetic retinopathy either in addition to photocoagulation or as a monotherapy (62).

2.3.3   Diabetic neuropathy

Diabetic neuropathy can be divided into three classes with multiple subgroups. Diffuse neuropathy includes distal symmetrical polyneuropathy and autonomic neuropathy (e.g., cardiac, gastrointestinal, and urogenital neuropathy). The other two classes of diabetic neuropathy are mononeuropathy and radiculopathy or polyradiculopathy (70). Distal symmetrical polyneuropathy and cardiac autonomic neuropathy are most studied, and after 20 years of diabetes duration the prevalence of distal symmetrical polyneuropathy and cardiac autonomic neuropathy is approximately 25–30% in people with type 1 diabetes (71).

Distal symmetrical polyneuropathy, in addition to peripheral artery disease, is an important cause of foot ulceration that can lead to lower-limb amputations. Therefore, annual foot screening, including evaluating the loss of protective sensation with monofilament testing, is recommended for all people with diabetes (62). Pharmacotherapeutic treatment options for diabetic neuropathy are mostly targeted on neuropathic pain, while the treatment options for other forms of neuropathy are limited (72). However, the electrophysiological abnormalities in nerve conduction related to diabetic neuropathy can be delayed or even prevented with optimal glycemic control (73).

2.4 Risk factors for vascular complications and atherosclerosis in type 1 diabetes

Risk factors for vascular complications can be categorized based on the mechanisms leading to either increased or decreased risk of different vascular complications. In addition to the risk factors seen in general population, people with type 1 diabetes have many diabetes related risk factors. **Figure 1** presents the categorization of vascular risk factors used in the following chapters, having a special emphasis on smoking and alcohol consumption and their impact on other risk factors.
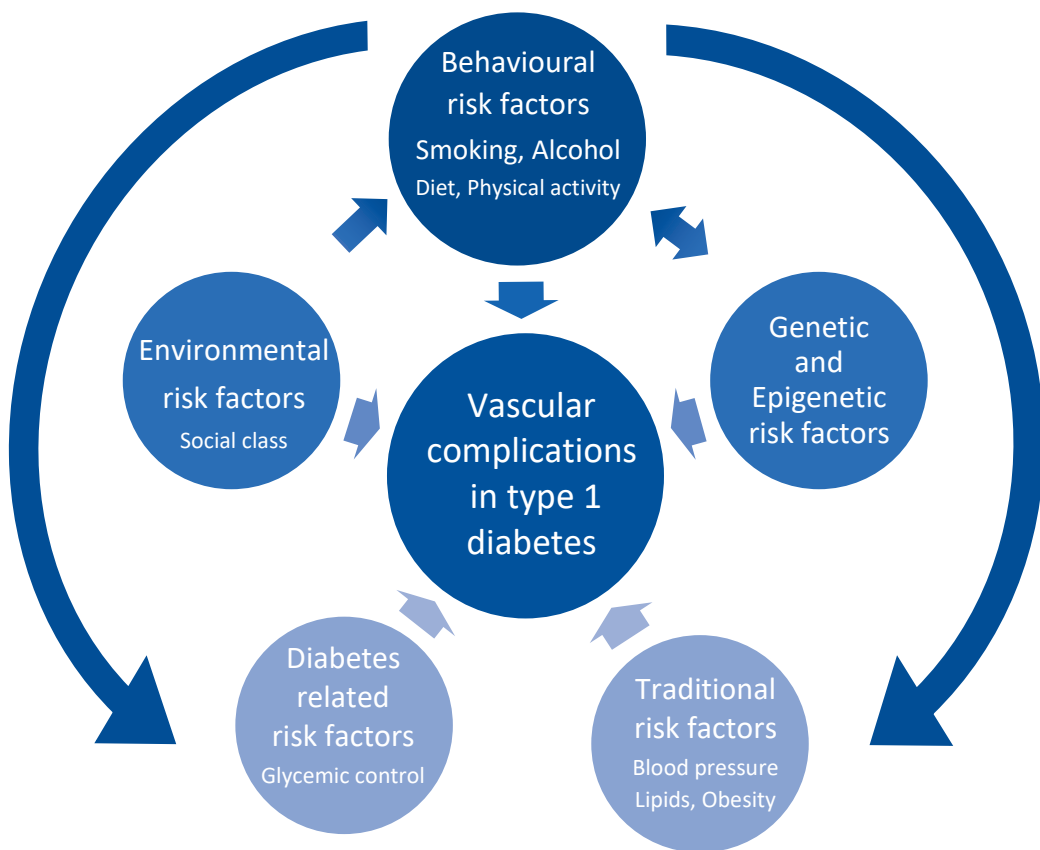
Figure 1. Interaction between smoking and alcohol and other risk factors for vascular complications in type 1 diabetes.

2.5 Traditional risk factors for vascular complications and atherosclerosis

2.5.1 Blood pressure

Hypertension is one of the most important risk factors for CVD. Based on a large meta-analysis, in people aged 40–69 years, each 20 mmHg difference in systolic blood pressure (SBP) or 10 mmHg difference in diastolic blood pressure (DBP), above 115/75 mmHg is associated with a 2-fold increase in the risk of CVD mortality (74). One study with 1.25 million people showed that blood pressure is associated with a significantly increased risk of all fatal or nonfatal CVD events and that the risk of CHD, heart failure, stroke, and peripheral artery disease increased 25–45% per each 20 mmHg increase in SBP (75). Globally, high SBP is the leading risk factor, causing 10.4 million deaths and 218 million disability-adjusted life years yearly (1). In Finland, the prevalence of hypertension (blood pressure ≥140/90 mmHg or the use of blood pressure medication) is 57.6% in men and 48.3% in women aged 30 or older(37).

Hypertension is also one of the major risk factors for the development of micro- and macrovascular complications in people with type 1 diabetes. The recent results from the Pittsburgh Epidemiology of Diabetes Complications (Pittsburgh EDC) study showed that hypertension was associated with an over 3-fold increased risk of any CVD and major atherosclerotic cardiovascular events (fatal or nonfatal myocardial infarction or stroke) during the 25-year follow-up (76). In the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC) study, the effect of blood pressure on the risk of CVD was significant but lower compared to the Pittsburgh EDC study results (77). In the DCCT/EDIC study, people with high blood pressure at baseline were excluded from the study, which might explain the difference between the studies. In the EURODIAB study, SBP was associated with the development of CHD but only significantly so in women (78). Based on the recent findings of the FinnDiane study, SBP was associated with an increased risk of both ischemic and hemorrhagic stroke, but DBP was only associated with an increased risk of hemorrhagic stroke (79).

Several observational studies on type 1 diabetes have shown that elevated blood pressure is associated with the development of microalbuminuria and macroalbuminuria (80-82). In addition, based on large clinical interventional studies, treating patients with microalbuminuria with angiotensin-converting-enzyme inhibitor or with an angiotensin II receptor blocker decreases progression to macroalbuminuria and increases regression to normoalbuminuria (83).

In the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), hypertension was strongly associated with the development of incident proliferative diabetic retinopathy (84). Other studies have shown an increased risk of diabetic retinopathy or its progression in people with higher SBP or DBP (69, 85, 86). Hypertension is also associated with the development of both distal symmetrical polyneuropathy and cardiac autonomic neuropathy in people with type 1 diabetes (87-89).

Based on the ADA recommendations, blood pressure targets in people with diabetes should be determined individually based on the overall cardiovascular risk profile. For people with diabetes and higher CVD risk (>15% in 10 years), the blood pressure target would be <130/80 mmHg, but with a lower CVD risk a target of <140/90 mmHg would be sufficient (90, 91).

2.5.2   Lipids

An elevated total cholesterol concentration is a risk factor for atherosclerosis and thromboembolic complications, such as myocardial infarction and ischemic stroke (92, 93). Low-density lipoprotein (LDL) cholesterol is accumulated in the intima layer of the arterial wall and causes plaque formation through an inflammatory process (94). High LDL cholesterol is particularly associated with a higher risk of CHD, but the association with ischemic stroke is still inconsistent (92, 95, 96). However, high-density lipoprotein (HDL) cholesterol is inversely associated with both CHD and ischemic stroke (92, 97, 98). Lowering LDL with statin treatment reduces the mortality and morbidity risk of all major vascular events, including a 20–30% reduction in CHD and a 22% reduction in ischemic stroke per each mmol/L LDL cholesterol (99, 100).

In general, the lipoprotein profile in people with type 1 diabetes is less atherogenic compared to that in people without diabetes, and the role of triglycerides seems to be more important in the development of CVD (101-103). In the DCCT/EDIC study, the strongest lipid parameters that predicted CVD were elevated triglycerides and LDL cholesterol (77). In the Pittsburgh EDC study, both HDL and non-HDL cholesterol were associated with the risk of CHD (104). Gender differences were discovered in the EURODIAB study, and HDL cholesterol was inversely associated with the risk of CHD in both men and women, but triglycerides were predictive of CHD only in women (78).

Diabetic nephropathy is associated with elevated total cholesterol, LDL cholesterol, and triglycerides and more atherogenic apolipoprotein-based profiles. In people with type 1 diabetes with normal UAER, instead of LDL cholesterol the ratios of atherogenic and

antiatherogenic lipoproteins and lipids are shown to be the strongest predictors of CHD (105). However, in people with macroalbuminuria, total and LDL cholesterol are predictive of CHD. Diabetic nephropathy not only alters the lipid profile but several lipid abnormalities, particularly elevated triglycerides, which also predict the progression of diabetic nephropathy in people with type 1 diabetes (106).

Based on an older study including participants with type 1 and type 2 diabetes, triglycerides are considered a risk factor for proliferative diabetic retinopathy (107). In a more recent study on type 1 diabetes, low HDL cholesterol and elevated triglycerides were associated with the risk of diabetic retinopathy (108). However, in a larger meta-analysis, including studies on type 1 and type 2 diabetes, only elevated total cholesterol was associated with diabetic macular edema but not with other types of diabetic retinopathy. A recent Mendelian randomization study on type 2 diabetes could not show any associations between the tested lipid fractions and diabetic retinopathy (69, 109). Many previous studies concerning the effect of statin therapy on the incidence or progression of diabetic retinopathy have had conflicting results (110). However, a recent large Taiwanese study showed that people with type 2 diabetes who were using statins had a 14% lower risk of incident diabetic retinopathy compared with a group not using statins (111). In the Fenofibrate Intervention and Event Lowering in Diabetes and Action to Control Cardiovascular Risk in Diabetes studies including patients with type 2 diabetes, triglyceride-lowering fenofibrate treatment was associated with a lower risk of diabetic retinopathy progression (112, 113).

Based on the EURODIAB study, elevated triglycerides, total cholesterol, and LDL cholesterol were all associated with the progression of diabetic neuropathy (peripheral or autonomic) (89). In the previously mentioned Taiwanese diabetic retinopathy study, statin treatment was also associated with a 15% lower risk of new-onset diabetic neuropathy (111). However, further research is needed to clarify the effect of statin treatment on the prevention of different forms of diabetic neuropathy, particularly in people with type 1 diabetes.

Based on the recent guidelines of the European Society of Endocrinology and the European Society of Cardiology, statin therapy is recommended for all adults with type 1 diabetes who have LDL cholesterol over 1.8 mmol/L (<70 mg/dL) and who are 40 or over or who have a duration of diabetes longer than 20 years or who have microvascular complications (114). In people with a very high risk of CVD, the LDL cholesterol target is even lower, namely 50% reduction and less than 1.4 mmol/L (<55 mg/dL). If the target is not achieved with statins, an additional LDL cholesterol-lowering therapy (ezetimibe or proprotein convertase subtilisin/kexin type 9 inhibitor) should be added to the treatment regimen (115).

2.5.3 Inflammation

Complex inflammatory pathways are involved in all phases of the atherosclerotic process—in early atherogenesis, in the progression of lesions, and in thromboembolic complications (116). Endothelial and inflammatory cells are activated, and numerous different pro-inflammatory cytokines, such as tumor-necrosis factor-α, interleukin-1β and interleukin-6 are involved in the process (116). Elevated levels of inflammatory markers, such as C-reactive protein (CRP) and fibrinogen, are associated with atherosclerosis and an increased risk of CVD (117-119).

In the Pittsburgh EDC study including 603 people with type 1 diabetes, the white blood cell count was associated with an increased risk of CHD (104). Several low-grade inflammatory markers and markers of endothelial dysfunction, such as CRP, interleukin-6, soluble vascular cell adhesion molecule, soluble E-selectin, plasminogen activator inhibitor 1, and fibrinogen are also associated with the development of diabetic nephropathy (120, 121).

Based on the WESDR study, soluble vascular cell adhesion molecule, tumor-necrosis factor, and elevated homocysteine levels were associated with more severe diabetic retinopathy in the presence of diabetic nephropathy. However, only homocysteine was associated with a higher risk of macular edema regardless of the diabetic nephropathy status (122). In the DCCT/EDIC study, baseline soluble E-selectin and plasminogen activator inhibitor 1 were associated with the development of diabetic retinopathy in the absence of other diabetic complications. However, many of the traditional inflammatory markers, such as CRP, tumor-necrosis factor receptors, and interleukin-6, were not associated with the development of diabetic retinopathy (123).

Hyperglycemia-induced low-grade inflammation and endothelial dysfunction are also associated with the development of diabetic neuropathy. The pathogenesis of distal symmetrical polyneuropathy is a complex network of biochemical mechanisms, including low-grade inflammation, endoplasmic reticulum stress, endothelial dysfunction, oxidative stress, and impaired mitochondrial function, all leading to neural damage (124).

Despite the increasing knowledge regarding the inflammatory process in the development of vascular complications, so far statins are the only medications used in clinical practice that have an anti-inflammatory effect in addition to lowering LDL cholesterol (125). However, a number of agents targeting different inflammatory pathways are being studied, and in the future some of them might be useful in preventing CVD (126).

2.5.4 Insulin resistance

Insulin resistance is defined as impaired insulin action in insulin-sensitive target tissues, such as skeletal muscle, adipose tissue, and the liver, leading to hyperglycemia, low-grade inflammation and dyslipidemia. Insulin resistance is an important predictor of the development of type 2 diabetes; it accelerates the progression of atherosclerosis and is causally associated with CVD events (127). Insulin resistance or sensitivity is traditionally measured by the glucose disposal (infusion) rate (GDR) using a euglycemic hyperinsulinemic glucose clamp test, or it can be estimated using methods such as the homeostasis model assessment or models based on an oral glucose tolerance test (128-130). In patients with type 1 diabetes, insulin sensitivity, can also be indirectly estimated based on an equation including the waist-to-hip ratio (WHR), history of hypertension, and the HbA_{1c} level, yielding an estimated glucose disposal rate (eGDR) (131). Lower eGDR values reflect lower insulin sensitivity (i.e., insulin resistance).

Although insulin resistance is a characteristic feature in people with type 2 diabetes, it is also commonly seen in people with type 1 diabetes (132, 133). Based on both the Pittsburgh EDC and the DCCT/EDIC studies, low eGDR is associated with an increased risk of CHD (104, 134). CVD risk is also elevated in people with type 1 diabetes who have a family history of type 2 diabetes, confirming the role of insulin resistance in the development of CVD (135).

Many studies have shown that insulin resistance is a risk factor for the development of diabetic nephropathy, and impaired insulin sensitivity is found in people with microalbuminuria, which partly explains the increased risk of CVD associated with diabetic nephropathy (134, 136-138). Increased insulin resistance also strongly correlates with a higher risk of diabetic retinopathy and neuropathy (89, 134, 139, 140).

The combination of type 1 diabetes and insulin resistance is often called "double diabetes", and some of the medications used in treating type 2 diabetes have also been tested in patients with type 1 diabetes with this particular condition. Although metformin, glucagon-like peptide-1 receptor agonists and SGLT2 inhibitors have some beneficial effects on body weight, lipid profile, HbA_{1c} values, and the insulin requirement, so far, the use of these medications has been limited to a selective group of patients (141-144) .

2.5.5   Obesity

The prevalence of obesity and overweight has been increasing globally during the last decades, causing excess morbidity and mortality. Based on WHO's Global Health Observatory data, 39% of the adult population and 18% of children and adolescents were overweight or obese in 2016 (145). Although the increase in adult obesity in developed countries has slowed, the prevalence of obesity among children is still growing, especially in developing countries (146, 147). Obesity is one of the major modifiable risk factors for CVD, and elevated BMI is associated with both fatal and nonfatal CHD and stroke (148, 149).

Obesity is traditionally measured by BMI, calculated as weight in kilograms divided by height in meters squared. The definition of obesity is BMI of ≥ 30.0 kg/m^2, while overweight is defined as BMI from 25.0–29.9 kg/m^2 (150). Regarding all-cause mortality, an optimal BMI seems to be 22.5–25.0 kg/m^2 and each 5 kg/m^2 higher BMI is associated with a 30% higher overall and 40% higher vascular mortality (151). However, based on the large INTERHEART study, WHR seems to have a stronger association with the risk of myocardial infarction compared with BMI, and the top two quintiles of WHR increase the population-attributable risk of myocardial infarction by 24.3% compared with only a 7.7% increased risk seen with the top two quintiles of BMI (152).

In people with type 1 diabetes, WHR, body weight, and BMI are all shown to be associated with the risk of CVD. However, the results have varied between studies, and gender differences have been reported. In an earlier report from the EURODIAB study, WHR was associated with a higher risk of CHD in men but not in women, and BMI was not a significant risk factor in that study (78). However, in a later CVD risk model analysis based on EURODIAB and two other cohort studies, WHR was a significant risk factor for major CVD outcomes, and each 0.1 unit increase in WHR increased the CVD risk by 30% (153). In a recent study from the Pittsburgh EDC, higher body weight and BMI were associated with the development of CVD in men but not in women (76).

In people with insulin-treated diabetes, weight gain and higher BMI are often associated with tighter glucose control, and therefore the effect on the risk of CVD related to weight gain might be different compared with the general population. In the DCCT/EDIC study, excess weight gain and obesity were associated with intensive treatment of glycemia. In a later report, excess weight gain was also associated with a higher coronary calcium score and intima media thickness, indicating a higher CVD risk

(154, 155). Excess weight gain should therefore be limited during intensive glucose treatment.

Obesity is also a risk factor for microvascular complications in people with type 1 diabetes. Both higher BMI and WHR are associated with the development of diabetic nephropathy, and higher BMI is associated with the development of diabetic retinopathy and neuropathy (86, 89, 156, 157). Based on the EURODIAB study, both body weight and BMI are associated with an increased risk of distal symmetric polyneuropathy (89).

In people with type 2 diabetes, weight loss (especially after bariatric surgery) might even lead to remission of diabetes and therefore to a reduced risk of macrovascular and microvascular complications (158). Based on a recent study including people with type 2 diabetes, bariatric surgery was associated with a significantly lower cumulative incidence of all-cause mortality, CHD, stroke, heart failure, atrial fibrillation and diabetic kidney disease compared with nonsurgical treatment (159). Studies have also shown a reduced risk of incident microvascular disease (diabetic nephropathy, retinopathy, and neuropathy) after bariatric surgery (160-162). Studies of bariatric surgery that have included people with type 1 diabetes are scarce but have shown favorable effects of weight loss on insulin requirement, glycemic control, blood pressure and lipid profile (163, 164). A small study including people with type 1 diabetes showed a potentially positive effect of bariatric surgery on diabetic nephropathy, while diabetic retinopathy remained mainly unaffected (165).

2.5.6   Age

Age is the strongest risk factor for any CVD. Globally, the prevalence of CHD is low in the younger age groups but starts to increase significantly after the age of 40. The prevalence of CHD is 3-fold higher in people aged 50–54 compared to those aged 40–44 (36). Similarly, the risk of stroke and peripheral artery disease increases with increasing age. In Finland, the prevalence of CHD increases from 5.2% in men aged 50–59 to 28% in men ≥80, and the corresponding percentages in women are 2.2% and 26.3% (37).

In studies of people with type 1 diabetes, age at onset of diabetes and diabetes duration are the more often used time variables instead of age. However, many studies have also reported data regarding the effect of age on the risk of different vascular complications. In the DCCT/EDIC study, age was the strongest risk factor for the

development of any CVD and major atherosclerotic cardiovascular event, with a linear association where every 5 years increased the risk of any CVD by 54% and increased the risk of major atherosclerotic cardiovascular event by 77% (77). The results from the EURODIAB study were similar, with a 93% increased risk of CVD per decade (153).

In people with type 1 diabetes, older age is also associated with the progression of diabetic nephropathy and a decline in kidney function measured by eGFR (166, 167). In the DCCT/EDIC study, age was associated with a higher risk of proliferative diabetic retinopathy and the risk increased by 1.4% per 1 year (168). A 10-year increase in age is associated with a 50% increase in the risk of incident cardiac autonomic neuropathy, and older age is also associated with the development of distal symmetric polyneuropathy (87-89).

2.5.7   Sex

2.5.7.1   *Sex differences in macrovascular disease*

Gender differences are observed in the risk of different cardiovascular outcomes. Atherosclerosis is rare in premenopausal women, but due to postmenopausal hormonal changes, the suboptimal primary and secondary prevention of CVD risk factors, and the longevity of women, the lifetime risk of CVD increases to a higher level in women compared to men (35, 169-171).

While women and men mostly share the same traditional cardiovascular risk factors, some differences were observed in the risk factor profile for acute myocardial infarction in the INTERHEART study. Hypertension, diabetes, physical activity, and moderate alcohol consumption were more strongly associated with myocardial infarction in women and former smoking in men. Other risk factors, such as dyslipidemia, current smoking, and obesity, had similar effects on the risk of myocardial infarction in both men and women (172). A history of pre-eclampsia and gestational diabetes are sex-specific risk factors for CVD seen in women. Pre-eclampsia doubles the risk of ischemic heart disease, and gestational diabetes increases the risk of any CVD by 70%, largely due to the increased risk of subsequent type 2 diabetes (173, 174).

Based on the recent Heart Disease and Stroke Statistics 2019 from the US, the prevalence of CHD is higher in men in all age groups compared with women; the total prevalence is 7.4% for men and 6.2% for women (35). Women are likely to suffer their first CVD event later than men; the average age for the first myocardial infarction is

65.6 for men and 72.0 for women. However, mortality after an acute myocardial infarction is higher in women compared with men in all age groups over 45 (35). The risk of heart failure is similar in men and women, although the cumulative lifetime risk is higher in women because of their higher life expectancy (35). Women also have a higher lifetime risk of any type of stroke than men, at 20–21% compared to 14–17%. The age-specific incidence rate of stroke is lower in women in the younger and middle-age groups but equal or higher in the oldest age groups (35). Based on the INTERSTROKE study, among the key risk factors for stroke, blood pressure and WHR were stronger for women and current smoking was stronger for men (4). The most important sex-specific risk factors for stroke in women are pre-eclampsia with an approximately 3-fold increased risk, gestational hypertension with a 60% increased risk, and the use of oral contraceptives with up to a 2-fold increased risk (175).

Based on a report published in 2016, the prevalence of self-reported CVD in Europe is 9.2%, the same for both sexes. However, in Finland the prevalence is higher in men at 13.0% compared with women at 10.9%. In Europe, the CVD mortality is higher in women, accounting for 49% of all deaths in women; this is compared with 40% of all deaths in men. Mortality in CHD is similar in both sexes, but women have a higher mortality in stroke and other forms of CVD. However, the premature mortality in CVD before 65–75 is clearly higher in men (34).

In the presence of diabetes, the protective effect of estrogen in premenopausal women is diminished, and women with type 2 diabetes have a 44% greater relative risk of fatal CHD compared with men (176). In type 1 diabetes, the CVD risk in premenopausal women is greater compared to women without diabetes, and the overall CVD risk seems to be similar compared to the CVD risk for men with type 1 diabetes (40). A recent meta-analysis of people with both type 1 and type 2 diabetes showed that diabetes is a stronger risk factor for stroke in women, increasing the relative risk by 27% compared to men (177). Based on the Nurses' Health Study, both type 1 and type 2 diabetes were associated with increased risk of stroke. However, the association was stronger in women with type 1 diabetes, with a 4-fold higher risk of stroke compared to women without diabetes. The risk in women with type 2 diabetes was 2-fold higher (46). Based on the Coronary Calcification in Type 1 Diabetes study, gender differences in insulin resistance-associated factors, such as WHR, waist circumference, and visceral fat distribution, could explain a part of the increased CVD risk seen in women with type 1 diabetes (178).

In people with type 1 diabetes, the risk of initial microalbuminuria and the development of macroalbuminuria is higher in men compared with women (82, 179). However, the sex difference in the risk of ESRD is modified by the age at onset of diabetes, and the risk is similar in men and women when diabetes is diagnosed early in life before age 10, but the risk is higher in men if the age at onset is 10 or older (180). Data regarding the association between sex and the development of diabetic retinopathy are conflicting. The large WESDR showed that men have a 33% higher risk of progression of diabetic retinopathy, but in some later studies the risk of diabetic retinopathy was similar in men and women (69, 84, 86, 181). Based on the FinnDiane study, also the risk of diabetic retinopathy is increased in men compared to women in association with increasing age at onset (180). In the EURODIAB study, there were no gender differences in the risk of developing distal symmetrical polyneuropathy or cardiac autonomic neuropathy in people with type 1 diabetes (88, 89).

2.6   Family history and genetics

A family history of CVD is a known risk factor for cardiovascular events. In the offspring cohort of the Framingham Heart Study, premature CVD (before the age of 55 in fathers and before the age of 65 in mothers) in at least one parent doubled the CVD risk in the offspring (182). Based on the same cohort, sibling CVD events increased the CVD risk by 50% (183). Previous studies have also shown familial clustering of diabetic nephropathy and proliferative diabetic retinopathy, suggesting a genetic component in the pathogenesis of these complications in people with type 1 diabetes (184, 185). For example, if one of the siblings in a sibling pair with type 1 diabetes has diabetic nephropathy, the risk in the other sibling is doubled (186).

In recent years, large GWAS and whole genome sequencing studies have found multiple gene loci associated with different vascular complications, such as CHD, diabetic nephropathy, and retinopathy (187-192). There are also some data regarding the association between genes and different types of diabetic neuropathy (193). Most of the genetic variants, single nucleotide polymorphisms (SNP), found in these studies are common and have only a small effect on the overall risk. However, these SNPs can be combined to construct genetic risk scores. In a study of CHD, a genetic risk score of nearly 50 000 SNPs improved the CHD risk prediction compared with traditional risk scores and demonstrated that a healthier lifestyle may compensate for the genetically

increased CHD risk (194). Genetic research can also lead to discoveries of new therapeutic agents to prevent vascular complications, such as the LDL cholesterol lowering proprotein convertase subtilisin/kexin type 9 antibodies that can decrease the LDL cholesterol up to 50–60% and reduce CVD morbidity by 15–25% (195, 196).

Based on current knowledge, genes alone account only for a small part of the risk of vascular complications. Recently, new technologies such as epigenomics, transcriptomics, proteomics, and metabolomics have provided new understanding of the link between genetic code and final functional consequences (197). Hopefully, in the future these data could be used to identify people at the highest risk of cardiovascular complications and to develop new therapeutic approaches for the treatment of vascular complications in people with diabetes.

2.7   Diabetes-related risk factors for vascular complications

Multiple studies have shown that people with type 1 diabetes have a 2–10-fold increased risk of CVD morbidity and mortality (198-200). Despite improvements in the treatment of cardiovascular risk factors and glycemia during the last 20 years, CVD mortality is still higher in people with type 1 diabetes compared with the general population (38, 201, 202). This is largely but not completely explained by the increased risk of CVD associated with diabetic nephropathy. However, glycemic control and other diabetes-related factors have also an effect on the development of CVD outcomes.

2.7.1   Duration of diabetes and age at onset of diabetes

Based on the DCCT/EDIC study, the risk of any CVD is increased by 25% and the risk of major atherosclerotic cardiovascular event by 33% per 5 years of duration of diabetes (77). A study based on the Swedish diabetes register, showed that early age at onset of type 1 diabetes (0–10 years) is associated with up to 5-fold increased excess CVD risk compared with later age at onset (26–30 years) (39).

In addition, the risk of microvascular complications is increased with the increasing duration of diabetes in people with type 1 diabetes. The risk of microalbuminuria is increased by 3.3% and the risk of macroalbuminuria is increased by 5.4% per one year of duration of diabetes (179). However, early age at onset has a protective effect on

the development of diabetic nephropathy, and prepubertal age at onset is associated with delayed onset of microalbuminuria (179, 203).

The prevalence of any diabetic retinopathy is higher with longer duration, being 21.1% with a duration <10 years compared to 76.3% with a duration ≥20 years (69). Based on the DCCT/EDIC study, an increase in the duration by one year increased the risk of different types of diabetic retinopathy; it increased proliferative diabetic retinopathy by 11.4%, clinically significant macular edema by 9.1%, and the necessity of ocular surgery by 8.9% (168). The association between age at onset and the risk of diabetic retinopathy is unclear. In the EURODIAB and FinnDiane studies, prepubertal age at onset was associated with a higher risk of incident proliferative diabetic retinopathy (204, 205). However, in other studies early prepubertal age at onset was associated with a lower risk of diabetic retinopathy (203, 206, 207).

The risk of developing distal symmetrical polyneuropathy increases with the duration of diabetes, and in people with type 1 diabetes who have a duration of ≥15.8 years the risk is almost 3 times higher compared with those who have a duration of <15.8 years (87). Based on the Pittsburgh EDC study, longer duration of diabetes is also a strong risk factor for diabetic autonomic neuropathy (208). The effect of age at onset on the development of diabetic neuropathy is seldom studied or separately reported, but in the Pittsburgh EDC study people who developed cardiac autonomic neuropathy during follow-up were younger at the time of diabetes diagnosis compared with those who did not develop neuropathy (7.9 vs. 9.3 years) (209).

2.7.2   Glycemic control

The association between glycemia and CVD risk in people with type 1 diabetes is unclear due to conflicting results in different studies. Many previous studies have not been able to show an association between glycemic control and the risk of CHD (78, 104, 210, 211). However, based on the latest data from the DCCT/EDIC study, the initial intensive treatment was associated with a 42% reduced risk of any CVD at 20 years of follow-up and a 30% reduced risk at 30 years. The risk of major cardiovascular events was reduced by 57% at 20 years of follow-up and by 32% at 30 years of follow-up. The difference was mainly due to the lower HbA$_{1c}$ values during the study period. However, the higher incidence of microalbuminuria and albuminuria in the conventional treatment group might explain part of the increased CVD risk (212, 213). In the DCCT/EDIC study, a 10% reduction (e.g., 7.2% vs. 8.0%) in HbA$_{1c}$ value during the 6.5-year study period was associated with a 20% reduction in the CVD risk during the 20

years of follow-up. In addition, later data from the EURODIAB and Pittsburgh EDC studies support the detrimental effect of poor glycemia on the risk of CVD in people with type 1 diabetes (153, 214).

Poor glycemic control is one of the key risk factors for the development of microalbuminuria and overt diabetic nephropathy in people with type 1 diabetes. Based on different studies, a 1% change in the HbA_{1c} value increases the risk of developing microalbuminuria by 13–18% (166, 179, 215). In the original Diabetes Control and Complications Trial (DCCT), intensive insulin therapy with close to normal glucose values (mean HbA_{1c} value around 7%) compared with conventional therapy (mean HbA_{1c} value around 9%) was associated with a 39% reduction in microalbuminuria and a 54% reduction in macroalbuminuria after 6.5 years of follow-up (216). Intensive insulin treatment has a long-term favorable effect on the development of diabetic nephropathy, and the difference between the two original DCCT treatment groups was still evident after 18 years of follow-up with a 51% reduction in the risk of ESRD in the intensive treatment group compared with conventional therapy (217, 218).

Glycemia is also one of the strongest risk factors for diabetic retinopathy. Based on the original results from the DCCT, after 6.5 years of follow-up intensive treatment of glycemia resulted in a 76% reduction of the risk of any incident diabetic retinopathy and a 54% reduction in the risk of three or more step progression of diabetic retinopathy. In addition, the risk of proliferative or severe nonproliferative diabetic retinopathy was reduced by 47% (216). This beneficial effect of intensive treatment was still observed 18 years after the original DCCT trial ended, with a 46% reduction in the risk of diabetic retinopathy progression in the former intensive treatment group compared with the former conventional treatment group, indicating a strong effect of metabolic memory on the development of diabetic retinopathy (85).

Based on the EURODIAB study, a 1% increase in HbA_{1c} is associated with a 36–44% increased risk of any diabetic neuropathy (89). In the DCCT study, combined peripheral sensorimotor and autonomic neuropathy was reduced by 60% in the intensive treatment group (216). The beneficial effect persisted, and the risk of incident peripheral neuropathy was 30% lower and the risk of cardiac autonomic neuropathy was 31% lower in the original intensive treatment group after the extended 14-year follow-up of the DCCT/EDIC (71). Most forms of diabetic neuropathy lack specific treatment, and therefore achieving good glycemic control is crucial in preventing neuropathic complications.

2.7.3 Microvascular complications and risk of other vascular complications

2.7.3.1 *Diabetic nephropathy*

Based on the DCCT/EDIC study, compared with people with type 1 diabetes who have normal UAER, microalbuminuria is associated with a nearly 2-fold increased risk of any CVD and macroalbuminuria is associated with a 2–3-fold increased risk (77). In the FinnDiane study, people with type 1 diabetes were compared with a control group without diabetes. The results showed that microalbuminuria was associated with a 6-fold increased risk of CHD, macroalbuminuria was associated with a 13-fold increased risk and ESRD was associated with an up to 27-fold increased risk (40). Similarly, the risk of stroke increased with the progression of diabetic kidney disease. Based on the DCCT/EDIC data, the risk of CVD seems to remain high even if once sustained microalbuminuria returns to normal UAER (219).

Diabetic nephropathy is also a risk factor for the development of diabetic retinopathy and neuropathy. Based on the DCCT/EDIC and WESDR studies, the risk of proliferative diabetic retinopathy is 2–2.5-fold higher in people with type 1 diabetes who have micro- or macroalbuminuria compared with normal UAER. Based on the EURODIAB study, UAER is also an independent risk factor for neuropathy, and when exceeding the level of macroalbuminuria the risk of any neuropathy is doubled (89).

2.7.3.2 *Diabetic retinopathy and neuropathy*

Diabetic retinopathy is associated with an increased risk of CVD. WESDR showed that the severity of diabetic retinopathy is associated with the risk of CHD (angina but not myocardial infarction), stroke, and cardiovascular mortality (210). In the EURODIAB study, both nonproliferative and proliferative diabetic retinopathy were associated with an increased risk of CVD, although the association was not independent but largely explained by traditional CVD risk factors (220). Based on a recent report from the FinnDiane study, severe diabetic retinopathy is associated with a nearly 50% increased risk of any CVD or CHD and a 90% increased risk of peripheral artery disease (221). Diabetic retinopathy is a strong risk factor for the development of other microvascular complications, and any diabetic retinopathy nearly doubles the risk of diabetic nephropathy (166). Any diabetic retinopathy also increases the risk of cardiac autonomic neuropathy by 70%, and proliferative diabetic retinopathy doubles the risk (88, 209). Cardiac autonomic neuropathy is associated with higher overall mortality and

an increased risk of several cardiovascular complications, such as left ventricular dysfunction, silent myocardial ischemia, mortality after myocardial infarction, and possibly even stroke (222-224). Diabetic neuropathy is also associated with an increased risk of diabetic nephropathy and retinopathy (215, 225).

2.8   Smoking and risk of vascular complications

Cigarette smoke is a mixture of more than 4000 different chemicals many of which are involved in the pathogenesis of atherosclerosis. Carbon monoxide and nicotine have acute effects on the vasculature through hypoxemia and vasoconstriction caused by activation of the sympathetic nervous system. Nicotine is also the main substance in cigarette smoke that causes dependency. However, other compounds such as different oxidants, play a more crucial role in the formation of atherogenic plaque, through endothelial dysfunction and injury, increased inflammation, and platelet activation (226).

2.8.1   Effect of smoking on vascular risk factors

2.8.1.1   Blood pressure

Based on experimental studies, smoking causes an acute increase in arterial wall stiffness and blood pressure that is mediated through catecholamine release caused by nicotine (227, 228). However, the long-term effect of smoking on the blood pressure is less clear. A large English population-based study found higher SBP only in older men who were heavy or moderate smokers compared with non-smokers (229). In contrast, in women there was a trend of lower blood pressure in light smokers. The association between smoking and blood pressure is also strongly affected by BMI and alcohol intake; therefore, the independent effect of smoking on blood pressure seems to be small. Experimental data of people with type 1 diabetes showed a higher 24-h ambulatory blood pressure in current smokers compared with non-smokers, possibly due to autonomic cardiac dysregulation (230).

2.8.1.2   Lipids

Smoking has detrimental effects on the serum lipid and lipoprotein concentrations. A comprehensive meta-analysis from 1989 showed that current smokers have higher serum concentrations of total cholesterol, triglycerides, very low-density lipoprotein and LDL cholesterol, and lower concentrations of HDL cholesterol and apolipoprotein A1 compared with non-smokers (231). The same meta-analysis showed a dose-response relationship between smoking and lipid metabolism, and a more atherogenic lipid profile is seen in heavy smokers compared with never smokers or light smokers (231). Smoking affects the lipid metabolism through different pathways, such as an increased catecholamine release that leads to increased free fatty acid concentration and increased very low-density lipoprotein formation (232). The triglyceride metabolism is affected by smoking through reduced lipoprotein lipase activity in skeletal muscle, leading to increased triglyceride levels. The altered triglyceride metabolism also affects the size of the LDL cholesterol particles leading to a lower and more atherogenic ratio of large to small LDL cholesterol particles. Smoking also induces oxidative stress, leading to lipid peroxidation and atherogenic plaque formation. HDL cholesterol, and particularly HDL_2 cholesterol, is reduced due to smoking through decreased lecithin cholesterol acyl-transferase activity and possibly also through increased cholesterol ester transfer protein and hepatic lipase activity. These altered metabolic mechanisms lead to dysfunctional HDL cholesterol and impaired reverse cholesterol transport into the liver (233).

In people with diabetes, the atherogenic effect of smoking on lipid values is similar to that in general population. Based on a meta-analysis of six studies combining people with type 1 and type 2 diabetes, non-smokers have lower LDL cholesterol and higher HDL cholesterol levels compared with current smokers (234).

2.8.1.3   Inflammation and hemostatic factors

Smoking is associated with increased inflammatory activation, measured by higher levels of CRP, white blood cells, interleukin-6, and fibrinogen in current smokers compared with never smokers (235, 236). In addition, people with type 1 diabetes who were active smokers had a higher leucocyte count compared with never smokers, indicating increased inflammatory activity (237). Smoking affects several stages of the coagulation and fibrinolytic pathways through increased levels of tissue factor,

thrombin, fibrinogen, and plasminogen activator inhibitor 1, leading to an increased risk of thromboembolic complications (226, 235).

2.8.1.4   Glucose metabolism and insulin resistance

In healthy people, smoking acutely impairs insulin action, probably due to a lower peripheral glucose uptake, leading to insulin resistance (238, 239). In people with type 1 diabetes, smoking is associated with poorer glycemic control, measured by higher HbA_{1c} levels in current smokers (240-244). Smoking is also associated with a higher daily insulin requirement, a sign of insulin resistance (245). Some studies have shown that smoking is associated with a lower risk of type 1 diabetes (246, 247).

The risk of type 2 diabetes is increased in a dose-response manner among smokers, probably due to the unfavorable effects on glucose metabolism (248-252). In a large meta-analysis including people with type 2 diabetes, HbA_{1c} levels were higher in both current and former smokers (253). However, there was no difference in fasting plasma glucose in current smokers compared with never smokers, and in fact the 2-h plasma glucose was lower in the current smokers. Therefore, the diagnosis of type 2 diabetes in current smokers might depend on the specific glycemic variable used as a diagnostic criterion. The prevalence of type 2 diabetes among smokers is higher if an elevated HbA_{1c} level is used for the diagnosis instead of the 2-h plasma glucose.

2.8.1.5   Obesity

Compared with non-smoking, current smoking is associated with lower body weight, lower BMI, and lower weight gain after the age of 25, in both men and women (254). However, there is some evidence that smoking is associated with increased abdominal fat and WHR; therefore, the overall cardiometabolic effect would be negative despite the lower BMI (255).

2.8.2 Gene-smoking interaction and DNA methylation

Candidate gene studies have conducted gene-smoking interaction analyses with previously known candidate gene loci for CHD. Seleheen et al. analyzed gene–smoking interaction at 50 loci associated with CHD risk and found that the 12% cardioprotective effect of the ADAMTS7 locus seen in never smokers was halved in current smokers (256). In another smaller study, a CHD risk allele was associated with an increased risk of CHD and CVD mortality only in never smokers, and the risk was attenuated in smokers (257). These results might be explained by the overall higher CVD risk in smokers, but direct changes in molecular and gene levels are also possible.

Another approach to evaluate gene–smoking interaction is to perform a genome-wide smoking–SNP interaction study. These GWAS have found novel loci for several CVD markers or risk factors, such as carotid intima-media thickness, coronary artery calcification, lipid variables, and blood pressure (258-261). A recent study addressed the interaction between a polygenic risk score for CHD and smoking (262). Based on the results, never smokers with the highest polygenic risk score had a similar risk of CHD compared to current smokers with the lowest polygenic risk score.

Epigenetic changes in deoxyribonucleic acid (DNA) methylation are one potential mechanism behind smoking exposure and different adverse health outcomes. Epigenetic studies have found approximately 2600 differentially methylated cytosine-phosphate-guanine sites (CpGs) in 1400 genes in current smokers compared with never smokers. These CpGs are also enriched in smoking-related diseases, such as CVD (263, 264). Smoking cessation leads to the normalization of methylation levels in most CpGs within 5 years of smoking cessation. However, nearly 200 CpGs remain differently methylated in former smokers compared with never smokers 30 years after smoking cessation, possibly explaining some of the permanent harm of smoking. Differences in gene methylation have also been used to design a methylation marker set that can identify smoking status, both current and former, from DNA samples (265). This information regarding smoking habits might be used in epidemiological studies in the future.

2.8.3 Smoking and mortality

The overall mortality is 3 times higher in smokers aged 25–79, compared with never smokers, and smoking is associated with a 10-year shorter life expectancy (266). Lung cancer mortality is around 15 times higher, and CVD mortality 2–3 times higher in smokers compared with never smokers (266). Based on a recent meta-analysis of people with diabetes, total and CVD mortality is 1.5 times higher in current smokers compared with never smokers (267). The risk seems lower than in the general population, but the difference is explained by the higher CVD mortality risk seen in all people with diabetes. Based on the same meta-analysis, in people with type 1 diabetes the total mortality risk is 1.8 times higher and CVD mortality 1.9 times higher in smokers compared with never smokers.

2.8.4 Smoking and cardiovascular disease

Cigarette smoking is one of the major risk factors for CHD. Based on the large INTERHEART study, current smoking was associated with a nearly 3 times higher risk of acute non-fatal myocardial infarction compared with never smoking, and the risk increases linearly with the increasing number of cigarettes smoked per day (2). In the INTERHEART study, the increased CHD risk associated with smoking was similar in men and women. However, in a large meta-analysis the risk of CHD associated with smoking was 25% higher in women compared with men (268). Smoking is also a strong risk factor for heart failure and current smokers carry a 2-fold increased risk of heart failure compared with never smokers (3, 269).

Based on the INTERSTROKE study, smoking is one of the five major risk factors that account for 80% of the global risk of all stroke, and in current smokers the risk of stroke (ischemic or hemorrhagic) is doubled compared with never smokers (4). In addition, the risk of stroke is increased with the number of cigarettes smoked per day, and people who smoke more than one pack (20 cigarettes) per day have over a 4-fold increased risk of stroke compared with never smokers (4). Regarding the risk of stroke, smoking is at least equally harmful for women as men, although there is some evidence of more harmful effects in women living in Western countries (270). In studies regarding both ischemic and hemorrhagic stroke, the majority of events are ischemic. However, separate studies regarding only hemorrhagic stroke events have shown an increased risk of total hemorrhagic stroke, intracerebral haemorrhage, and

subarachnoid hemorrhage in current smokers compared with never smokers (271, 272).

In people with diabetes (type 1 and type 2 combined), current smoking is associated with approximately 50% higher risk of CHD and stroke compared with never smoking (267). Among people with type 1 diabetes, the associations between smoking and different CVD disease entities have been studied to a lesser extent, and specific dose-response data are lacking. In addition, many studies have only addressed CVD mortality or combined CVD and not specific CVD events, and the results have been conflicting (273, 274). Few studies have shown an increased CHD risk in ever smokers compared with never smokers (104, 275). But only in one study was the risk of non-fatal CHD higher in former smokers (210). Other studies, including the EURODIAB study, have not been able to show significant associations between smoking and the risk of CHD (78, 274).

Only two studies have reported findings regarding the association between smoking and heart failure in people with type 1 diabetes. A larger study based on the Swedish national diabetes registry showed that smoking was associated with an increased risk of heart failure but only when a person was registered as a smoker in more than 50% of the registration events (276). In a smaller Polish study, smoking was not associated with an increased risk of heart failure diagnosed by echocardiography of each study subject (277).

In the general population, smoking is strongly associated with peripheral arterial disease, and the risk of intermittent claudication is nearly 4-fold higher in heavy smokers (>25 pack-years) compared with never smokers (278-280). In people with type 1 diabetes, smoking is associated with a 2-fold risk of ulcers and heavier smoking is also associated with the risk of lower extremity amputations, with a 30% increased risk per 10 pack-years of smoking (281, 282).

2.8.5   Smoking and microvascular complications

2.8.5.1   *Diabetic nephropathy*

In the general population, current smoking is associated with a 2–4-fold increased risk of ESRD or death due to chronic kidney disease compared with non-smokers (283, 284). The nephrotoxic effect of cigarette smoke is mediated through many different mechanisms, such as hypoxia, oxidative stress, prothrombotic factors, pro-

inflammatory cytokines, intrarenal vasoconstriction, and nicotine-induced cell proliferation (285). These mechanisms lead to tubular damage and glomerular sclerosis and eventually to a decline in kidney function.

In addition, in people with diabetes smoking is associated with a decline in kidney function measured by eGFR (286). However, results from studies regarding the association between smoking and the progression of diabetic nephropathy have been conflicting. Older studies with a cross-sectional design or only a short follow-up showed that smoking was associated with proteinuria in people with type 1 diabetes (241, 287-291). In a Danish study with 10 years of follow-up, current smoking was associated with a higher risk of developing micro- and macroalbuminuria (166). However, most of the later prospective studies with longer follow-up have not confirmed the association between smoking and the progression of diabetic nephropathy (82, 215, 244, 286, 292). These studies also lack data regarding cumulative smoking in pack-years and intensity of smoking in packs per day; therefore, the results are limited to simple smoking status.

2.8.5.2   *Diabetic retinopathy and neuropathy*

The results regarding the effect of smoking on the risk of diabetic retinopathy or neuropathy have varied during different time periods. An earlier cross-sectional study from the 1980s reported a positive association between current smoking and the prevalence of proliferative diabetic retinopathy in people with type 1 diabetes (287). An earlier cross-sectional report from the EURODIAB study showed an association between current and ex-smoking and diabetic retinopathy in men (241). However, after 7.3 years follow-up, current smoking was not associated with the incidence of proliferative diabetic retinopathy in the EURODIAB study (204). The results from the DCCT/EDIC study were similar to the prospective EURODIAB results, and smoking was not associated with the development of proliferative diabetic retinopathy after more than 30 years of follow-up (168). In an early report from the Pittsburgh EDC study, ever smoking was associated with an increased risk of diabetic autonomic neuropathy, but the finding was not confirmed in the later report from the same study or in the EURODIAB study (88, 208, 209). However, ever smoking is shown to increase the risk of distal symmetrical polyneuropathy by 70% (87).

2.8.6   Smoking cessation

While active smoking is associated with the deterioration of many cardiometabolic risk factors, some but not all are improved after smoking cessation. Smoking cessation is often associated with weight gain that occurs rapidly during the first months after smoking is stopped. The mean body weight increase at one year after smoking cessation is 4–5 kg, but the inter-individual variation is wide (293). Even though >5 kg weight gain after smoking cessation is associated with a higher risk of type 2 diabetes, the risk of all-cause and CVD mortality is still reduced in all former smokers compared with current smokers (294). Based on experimental studies, smoking cessation can within a few weeks acutely improve insulin sensitivity. However, after a few months insulin sensitivity deteriorates, probably due to weight gain (295, 296). In people with type 2 diabetes, HbA_{1c} is increased during the first 1–2 years after smoking cessation, but after that glycemic control improves and by 3 years the HbA_{1c} level is similar to that of continual smokers (297). Similar studies of people with type 1 diabetes investigating the effect of smoking cessation on glycemic control compared to continual smoking do not exist. However, in the EURODIAB study, the HbA_{1c} level was similar in never smokers compared with former smokers and higher in current smokers (241). Smoking is associated with a more atherogenic lipid profile, which is improved after smoking cessation. Despite weight gain, HDL cholesterol is significantly increased after smoking cessation, but total cholesterol, LDL cholesterol, and triglycerides are not affected (298, 299). There is also evidence that the inflammatory process related to smoking is attenuated by smoking cessation (300, 301).

In the general population, smoking cessation has a clear beneficial effect on all-cause mortality (266). If smoking is stopped at the age of 25–34, the mortality risk is similar to that in never smokers. However, smoking cessation later in life is also beneficial, and if smoking is stopped at the age of 55–64, 4 years of life are gained compared with people who have continued to smoke. In addition, the risk of CHD is decreased after smoking cessation, but based on the INTERHEART study the risk of acute myocardial infarction is still 22% higher more than 20 years after quitting in former smokers compared with never smokers (2). In former smokers who have stopped smoking >15 years earlier and who smoked less than 32 pack-years, the risk of heart failure is similar compared with never smokers (302). In the INTERSTROKE study, the risk of stroke in former smokers decreased even below the risk seen in never smokers (4). However, based on a large meta-analysis, the risk of any stroke in former smokers is 17% higher in women and 8% higher in men compared with never smokers, and the risk is lower compared with current smokers in both men and women (270). The risk of peripheral

artery disease is not decreased after smoking cessation to the same degree as the other CVD outcomes, and the risk of peripheral artery disease is still 2-fold higher in former smokers compared with never smokers (49).

Some of the older studies have reported a favorable effect of smoking cessation on UAER values in people with type 1 diabetes (288, 289). However, the EURODIAB study showed contrary results, and the prevalence of macroalbuminuria was the highest in men who were former smokers (241). Studies regarding the effect of smoking on the risk of diabetic nephropathy have often combined former smokers with never or current smokers, and therefore the effect of smoking cessation on development of diabetic nephropathy remains largely unclear (166, 215, 244). As the results regarding the association between smoking and the risk of diabetic retinopathy and neuropathy are conflicting, there is no clear evidence regarding the effect of smoking cessation on the development of these complications either.

2.8.7 Dose-dependent measures of smoking

Traditionally, the dose-dependent analyses regarding smoking and CVD risk have included pack-year data. However, the cumulative dose can also be calculated by converting the duration of smoking and the intensity of smoking (packs per day) into pack-years. A recent epidemiological study compared the effect of the cumulative dose of pack-years with the intensity of smoking measured in packs per day on the risk of CVD (303). Based on the findings, it seems that the intensity of smoking might be a better measure of smoking-related CVD risk compared to pack-years or plain smoking status. Both the INTERHEART and INTERSTROKE studies showed a linear association between the intensity of smoking (cigarettes per day) and the risk of CHD and ischemic stroke (2, 4). Previous studies regarding the risk of CVD in people with type 1 diabetes do not provide more accurate data on the effect of cumulative smoking and intensity of smoking.

2.8.8 Second-hand smoke

Exposure to second-hand smoke or passive smoking is associated with a 25% increased risk of CHD and stroke (304, 305). During the last decades, several actions have been taken regarding Finnish tobacco legislation to reduce the harmful effects of second-hand smoke. The act for smoke-free workplaces was introduced in 1994, and smoking

in restaurants was banned in 2007. The prevalence of people exposed to second-hand smoke at their work-place has declined from 24% in 1994 to 4% in 2014, when the data regarding second-hand smoke were last collected in Finland (306).

2.9 Alcohol and risk of vascular complications

Unlike the harmful effect of smoking across the different disease entities, the effect of alcohol consumption is more complex. The detrimental effect of alcohol consumption leading to the increased risk of many different forms of cancer, liver cirrhosis, and injuries is well established. However, based on numerous epidemiological studies, light-to-moderate alcohol consumption is associated with beneficial effects on atherosclerotic vascular diseases, particularly CHD. Like smoking, alcohol consumption affects vasculature through many known cardiovascular risk factors and atherogenic pathways.

2.9.1 Effect of alcohol consumption on cardiovascular risk factors

2.9.1.1 *Blood pressure*

Alcohol consumption is associated with increased blood pressure and experimental studies have shown a rapid decrease in the blood pressure after the cessation of alcohol consumption (307). A large meta-analysis of clinical trials studying the effect of reduced alcohol consumption on blood pressure reported a -3.31 mmHg reduction in the SBP and a -2.04 mmHg reduction in the DBP when the mean baseline alcohol consumption was 3–6 drinks per day and the average reduction of daily consumption -67% (308). In a study of people with hypertension and alcohol dependency the effect of alcohol abstinence was even stronger; after a 16-week treatment period SBP decreased 12 mmHg and DBP decreased 8 mmHg (309). Alcohol consumption beyond two drinks per day is associated with an increased incidence of hypertension in both men and women (310). The most recent American Heart Association guideline for high blood pressure recommends alcohol consumption ≤2 drinks per day for men and ≤1 drink per day for women to minimize the harmful effect of alcohol on blood pressure (311).

2.9.1.2 Lipids

Alcohol consumption has well-known effects on lipids. Based on a meta-analysis of the effect of alcohol consumption on lipids and hemostatic factors, the largest dose-dependent effect was on HDL concentration. With an average alcohol consumption of 30 g (2.5 drinks) per day, HDL cholesterol was 3.99 mg/dl (0.10 mmol/l) higher compared with abstainers (312). A smaller increase was also reported for the concentrations of triglycerides and apolipoprotein A1. A more recent meta-analysis of the effect of moderate alcohol consumption on lipids reported a significant increase only in HDL cholesterol (0.09 mmol/l); there was no effect on total cholesterol, LDL cholesterol, or triglycerides (313). However, higher alcohol intake per drinking session (≥5 drinks) has been shown to elevate triglyceride concentrations (≥150 mg/dl or 1.7 mmol/l) in both men and women (314).

2.9.1.3 Inflammation and hemostatic factors

Some studies have reported a lower CRP in moderate alcohol consumers compared with abstainers and heavy consumers (315, 316). However, this possible anti-inflammatory effect of moderate alcohol consumption was not fully confirmed in a meta-analysis where no significant associations between alcohol consumption and CRP, interleukin-6, or tumor-necrosis factor α were found (313). Smaller studies have also shown associations between alcohol consumption and different hemostatic markers, such as fibrinogen, D-dimer, and plasminogen activator inhibitor 1 (316, 317). The strongest effect is on fibrinogen, with a reduction of -0.20 g/l in moderate consumers (313).

2.9.1.4 Glucose metabolism and insulin sensitivity

Moderate alcohol consumption is associated with an increase in adiponectin, which could lead to improved insulin sensitivity through the suppression of glucose production in the liver and increased glucose uptake and fatty acid oxidation in the muscles (313, 318). Based on a meta-analysis of intervention studies, moderate alcohol consumption decreased fasting insulin and HbA_{1c}, but no significant effect was seen on the fasting glucose concentration or insulin sensitivity, except a trend toward increased insulin sensitivity in women (319). Moderate alcohol consumption is associated with a

lower risk of type 2 diabetes, but based on the latest evidence this association is only seen in women, with a peak risk reduction of 18% with an alcohol consumption of 1 drink per day (320).

In people with type 1 diabetes, alcohol is associated with an increased risk of hypoglycemia, and the decrease in glucose is seen 8–12 hours after alcohol intake. Alcohol consumption may also impair cognitive function and therefore blunt hypoglycemia awareness. At the molecular level, alcohol suppresses growth hormone levels leading, to impaired gluconeogenesis and hypoglycemia (321).

2.9.2   Alcohol consumption and cardiovascular disease

Alcohol consumption influences the development of CVD through complex pathways, including the modification of the above-mentioned traditional risk factors and a variety of interactions at the cellular and molecular levels (322). Based on multiple observational and interventional studies, moderate alcohol consumption seems to have a protective effect on some CVD entities. In a large meta-analysis, alcohol consumers had 25% reduced CHD mortality and a 27% reduced risk of incident CHD compared with life-long abstainers (323). The risk of CHD morbidity and mortality is lowest with a consumption of 2–3 drinks per day in men and 1 drink per day in women (324). In men, the CHD mortality risk increases with an increasing amount of alcoholic drinks, but the CHD morbidity risk seems to remain similar, even with higher consumption. However, in women not only the CHD mortality risk but also the morbidity risk increases with increasing alcohol consumption and in a steeper manner than in men. In former drinkers, the risk of CHD morbidity is similar to that in life-long abstainers, but CHD mortality is significantly higher (323, 325).

Regarding the risk of stroke, the protective effect of alcohol is clearly smaller and only seen for the risk of ischemic stroke and not for intracerebral hemorrhage or subarachnoid hemorrhage (326). Alcohol consumption of ≤2 drinks per day is associated with an 8–10% lower risk of ischemic stroke compared with abstainers, but the risk is significantly higher when alcohol consumption exceeds the limit of 2 drinks per day. Moderate alcohol consumption has no significant effect on the risk of hemorrhagic stroke. However, the risk of hemorrhagic stroke is increased with increased alcohol consumption, and heavy drinking (>4 drinks per day) is associated with a 67% higher risk of intracerebral hemorrhage and an 82% higher risk of subarachnoid hemorrhage (326).

There are no data regarding alcohol consumption and CVD risk in people with type 1 diabetes, but a few studies of people with type 2 diabetes have shown an association between alcohol consumption and a lower risk of CHD. Based on a small meta-analysis of six studies, alcohol consumption reduced the risk of total and fatal CHD by 25–66% (327). In women with type 2 diabetes, light-to-moderate alcohol consumption is associated with a 50% reduction in the risk of fatal or nonfatal CHD (328). In men, the results are similar, and regular alcohol consumption or the consumption of >2 drinks per day are associated with a lower CHD risk compared with abstainers (329, 330). The Action in Diabetes and Vascular Disease (ADVANCE) trial reported that moderate alcohol consumption was associated with a 17% lower combined CVD risk, including stroke, but no specific data regarding the association between alcohol consumption and stroke in people with type 2 diabetes are available (331).

2.9.3   Alcohol consumption and microvascular complications

Only a few previous studies have addressed the association between alcohol consumption and microvascular complications in people with diabetes. Based on the EURODIAB study, compared with abstainers, moderate alcohol consumers had a lower risk of macroalbuminuria, proliferative diabetic retinopathy, and neuropathy (332). However, some diabetic retinopathy studies have shown contradictory findings, with an increased risk of diabetic retinopathy in either light or heavy consumers (107, 333). An early cross-sectional report from the WESDR showed a possible beneficial effect on the risk of diabetic retinopathy in people with type 1 diabetes, but their later prospective study did not support these earlier findings (334, 335). In the ADVANCE trial including people with type 2 diabetes, alcohol consumption was associated with a 15% reduction in the risk of microvascular complications (diabetic nephropathy or retinopathy combined) (331). Based on recent data from the DCCT/EDIC study, occasional or regular drinking was not associated with the risk of incident proliferative diabetic retinopathy (168). Due to the conflicting results of the different diabetic retinopathy studies and the rather scarce data with respect to diabetic nephropathy, the overall effect of alcohol consumption on the risk of diabetic retinopathy and nephropathy remains unclear.

2.9.4 Effect of drinking pattern and beverage type

Even if light-to-moderate alcohol consumption might have a beneficial effect on some CVD outcomes, the effect is strongly influenced by the drinking pattern. Irregular heavy drinking occasions, >60 g or ≥5 drinks per occasion at least monthly, are associated with up to a 45% increased risk of CHD compared with regular moderate drinking (336). Based on a Finnish study, binge drinking increases the risk of any stroke by 85%, and the risk of ischemic stroke is nearly doubled compared to non-binge drinkers (337). Based on an older review study that collected data from 10 large prospective cohort studies, there was no consistent pattern indicating that any specific beverage type (wine, beer, or spirits) would have a more beneficial effect on the risk of CHD (338). However, later meta-analyses of the effect of wine, beer, and spirit consumption on the risk of CVD have reported a protective effect associated with both moderate wine and beer consumption, but no significant protective effect was associated with spirit consumption (339, 340). In the EURODIAB study, moderate wine consumption was associated with a lower risk of both diabetic nephropathy and retinopathy (332). It also reported a lower diabetic retinopathy risk in moderate beer consumers but did not find any association between spirit consumption and the risk of microvascular complications. Based on the ADVANCE trial, in people with type 2 diabetes different beverage types were not associated with the risk of diabetic retinopathy (341).

2.9.5 Relationship of alcohol consumption with other environmental risk factors

Moderate alcohol consumption and physical activity are both shown to be associated with an overall healthier lifestyle (342). There are also differences in environmental risk factors between drinkers of different beverage types. Wine drinkers seem to have higher socioeconomic status, measured by education and income (343). A Danish study showed that people who buy wine also have healthier food-buying habits compared with people who buy beer (344). In another Danish study, wine drinking was associated with better psychological functioning and higher IQ scores compared with beer drinkers (345). A Finnish study reported similar findings, with better self-reported health, higher self-efficacy, and less psychological distress in people who regularly drink wine with meals (346). Therefore, these many behavioral and socioeconomical characteristics that correlate with the choice of drink might largely explain the differences seen in the association between beverage type and CVD risk (347).

3 AIMS OF THE STUDY

The specific aims of this thesis were as follows:

I   To examine the cross-sectional association between the amount of alcohol consumption and the type of beverage and the risk of diabetic nephropathy and severe diabetic retinopathy in people with type 1 diabetes.

II   To evaluate the effect of cumulative smoking in pack-years on the development of diabetic nephropathy in people with type 1 diabetes.

III   To evaluate the combined effect of smoking and the rs4972593 gene variant on the development of end-stage renal disease in people with type 1 diabetes.

IV   To investigate the association between cumulative smoking in pack-years and the intensity of smoking in packs per day and the risk of coronary heart disease, heart failure and stroke in people with type 1 diabetes.

4 SUBJECTS AND STUDY DESIGN

These studies are part of the ongoing FinnDiane study, a nationwide, multicenter, prospective study with the aim of identifying the clinical, genetic, and environmental risk factors for micro- and macrovascular complications in people with type 1 diabetes. The FinnDiane study was officially launched in 1997, although some participants were recruited during the two pilot studies (GENREL and NEFREL) during 1994–1996. The participants were recruited at their regular visits to the outpatient clinics at each study center (all 5 Finnish university hospitals, 16 central hospitals, 26 regional hospitals, and 30 primary health care units). Although the FinnDiane study is not strictly population-based, the distribution of the participants is similar to the distribution of the general population in Finland. The recruitment criteria for type 1 diabetes were age <40 when the diagnosis was made and initiation of insulin treatment within one year of the diagnosis.

Currently, 5500 people with type 1 diabetes have been recruited to the FinnDiane study. A prospective phase of the study was started in 2004 covering approximately 1900 participants. Follow-up data were also obtained from medical files and regular updates of national registers, such as the Care Register for Health Care and the Causes of Death Register. All participants have given their informed written consent. The local ethics committees have approved the study protocol, and the study is carried out in accordance with the principles of the Declaration of Helsinki.

4.1 Study I

The study design of the first study is cross-sectional. This study comprises 4187 participants who had enrolled in the FinnDiane study by the end of 2008 with known renal or diabetic retinopathy status. A total of 579 participants were excluded because of unclear data regarding their exact alcohol consumption, and 141 were excluded because of unclear data regarding the of type beverage consumed. Data regarding alcohol consumption in g per week were available for 3608 participants, and data regarding the type of beverage consumed were available for 3467 participants (including abstainers). A more detailed description of the study population is given in **Table 3**.

4.2 Study II

The second study is a prospective study that included 4269 participants, with known renal status at baseline who had enrolled in the FinnDiane study by the end of 2012. Exclusion criteria were prevalent ESRD at baseline (359 participants), unclear data regarding smoking status (293 participants), unclear data regarding smoking in pack-years (202 participants), and unclear follow-up data regarding renal status (4 participants). The final cohort comprised 3613 participants with known smoking status and 3411 participants with known pack-year data. The baseline characteristics of the study population are given in **Table 3**.

4.3 Study III

The third study is a prospective study that included 4269 participants who had enrolled in the FinnDiane study by the end of 2012 with known renal status. Participants with unclear data regarding smoking status (338), unclear data regarding the risk allele of interest (1306 participants), and unclear follow-up data regarding ESRD (4 participants) were excluded from the study. The final cohort comprised 2621 participants. Patient characteristics of the study population are presented in **Table 3**.

4.4 Study IV

The fourth study is a prospective study that included 4771 participants who had enrolled in the FinnDiane study by the end of 2013. Participants with unclear smoking status (261 participants), unclear data regarding smoking in pack-years (252 participants), unclear data regarding smoking intensity (83 participants), or unclear data regarding the follow-up of CVD outcomes (4 participants) were excluded. The final cohort comprised 4506 participants with known smoking status, 4254 participants with known pack-year data, and 4423 participants with known data regarding the intensity of smoking in packs per day. The study population is described in more detail in **Table 3**.

Table 3. Participant characteristics at baseline

Characteristic		Study I	Study II	Study III	Study IV
N (Men %)		3608 (52.6)	3613 (51.1)	2621 (50.2)	4506 (51.9)
Age, years		37.4 (28.9–46.8)	36.6 (28.0–46.2)	41.0 (32.4–48.9)	38.2 (29.1–47.5)
Duration of diabetes, years		21.2 (12.1–30.6)	20.1 (11.3–29.9)	25.8 (18.7–33.4)	21.5 (12.3–31.1)
HbA$_{1c}$, mmol/mol (HbA$_{1c}$, %)		68.6±16.1 (8.4±1.5)	68.8±16.2 (8.4±1.5)	69.5±15.7 (8.5±1.4)	68.7±16.1 (8.4±1.5)
Systolic blood pressure, mmHg		134±19	133±18	137±20	135±19
Diastolic blood pressure, mmHg		79±10	79±10	80±10	79±10
Total cholesterol, mmol/l		4.94±1.04	4.90±0.95	5.03±0.98	4.92±0.99
LDL cholesterol, mmol/l		3.04±0.94	3.01±0.84	3.13±0.86	3.04±0.86
HDL cholesterol, mmol/l		1.33±0.39	1.34±0.38	1.32±0.39	1.34±0.39
Triglycerides, mmol/l		1.03 (0.78–1.47)	1.01 (0.76–1.42)	1.05 (0.78–1.51)	1.04 (0.78–1.48)
Social class, two highest classes (%)		31.4	31.8	32.9	31.7
Smoking status (%)	Never	52.7	53.9	51.8	51.8
	Current	24.1	25.8	24.1	25.5
	Former	23.2	20.3	24.1	22.7
Alcohol consumption (%)	Abstainers	23.8	23.2	24.5	23.7
	Light	46.9	48.1	46.9	46.8
	Moderate	22.1	22.1	21.0	21.8
	Heavy	3.3	3.2	3.1	3.4
	Former	3.9	3.4	4.5	4.3
Renal status (%)	Normoalbuminuria	62.6	69.8	53.6	61.4
	Microalbuminuria	12.7	14.5	15.5	12.9
	Macroalbuminuria	13.9	15.8	20.2	14.2
	ESRD	6.3	-	10.6	7.5
	Unclassified	4.5	-	-	4.1
Severe diabetic retinopathy (%)		33.2	29.4	45.6	34.3

Data are expressed as mean ± standard deviation, median (interquartile range) or (%). ESRD: end-stage renal disease.

5 METHODS

5.1 Anthropometric measurements, body mass index, and blood pressure

During the study visit, body weight was measured while wearing light clothing and registered to the closest 0.1kg, and height was registered to the closest 1 cm. BMI was calculated as body weight divided by height squared (kg/m^2). Waist circumference was measured midway between the lowest rib and the iliac crest and, hip circumference was measured at the level of the major trochanters of the femurs. WHR was calculated by dividing the waist circumference by the hip circumference. Blood pressure was measured twice at 2-min intervals in a supine position after a 10-min rest using a mercury sphygmomanometer or an automated standardized blood pressure device. The mean of these measurements was then calculated and used in the analyses. Hypertension was defined as SBP >140 mmHg or DBP >90 mmHg or known blood pressure medication based on the Drug Prescription and Drug Reimbursement Registers.

5.2 Laboratory measurements

HbA_{1c} values were measured locally at each study center by standardized assays and were reported in both mmol/mol and percentages. Insulin sensitivity was determined by the eGDR, that was calculated with an equation modified for the use of HbA_{1c} instead of HbA_1: eGDR = 24.4 − 12.97 x WHR − 3.39 x AHT − 0.60 x HbA_{1c}, where AHT stands for antihypertensive treatment or blood pressure ≥140/90 mmHg (yes = 1, no = 0) (131). Serum lipids and lipoproteins were measured centrally in the Professor Marja-Riitta Taskinen's laboratory at the research laboratory of the Helsinki University Central Hospital. Serum creatinine concentrations were also measured centrally from the blood samples at the laboratory of the Helsinki University Central Hospital, Helsinki, Finland.

5.3 Definition of diabetic nephropathy and assessment of renal function

The diagnosis of diabetic nephropathy was defined as macroalbuminuria (albumin excretion rate >200 µg/min or >300 mg/24 h) or ESRD. UAER was determined at a central laboratory from timed overnight or 24-h urine collections by radioimmunoassay (Pharmacia, Uppsala, Sweden) until 2002 and thereafter by an immunoturbidimetry method (Hitachi 911 analyzer, Roche Diagnostics, Hoffman-La Roche, Basel Switzerland). Based on at least two out of three UAER measurements, the participants were divided into three different classes: normoalbuminuria (UAER less than 20 µg/min or 30 mg/24 h), microalbuminuria (UAER ≥ 20 <200 µg/min or ≥30 <300 mg/24 h), and macroalbuminuria (UAER ≥200 µg/min or ≥300 mg/24 h). ESRD was defined as participants receiving dialysis treatment or having undergone kidney transplantation. Any progression in diabetic nephropathy included progression from normoalbuminuria to microalbuminuria, from normoalbuminuria or microalbuminuria to macroalbuminuria, or from a lower renal stage to ESRD. Renal function was estimated by eGFR calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (54).

In Study I, the diabetic nephropathy status was defined at baseline. In Study II and Study III, the follow-up data for diabetic nephropathy and ESRD were based on the medical records and on the Finnish Care Register for Health Care maintained by the National Institute for Health and Welfare that includes all dates for hospital admissions and discharges and diagnoses for the care periods. Follow-up data were also obtained from the Cause of Death Register based on the data from death certificates. For ESRD, the following International Classification of Diseases (ICD) codes were used: ICD-10: Z940, T824, Z992, Y841 and T861 (http://apps.who.int/classifications/icd10/browse/2016/en) and the following procedure codes based on the Nordic Medico-Statistical Committee: KAS10, KAS20, KAS40, KAS60, KAS61, TJA33, TJA35, TK800 and TK820 (since 1996 http://nordclass.se/ncsp_e.htm).

5.4 Definition of severe diabetic retinopathy

Diabetic retinopathy status was recorded during baseline visits using questionnaires completed by a health care professional (attending physician or diabetes nurse). The questionnaires included data regarding the level of diabetic retinopathy and the time of diagnosis and possible laser treatment. In Study I, severe diabetic retinopathy was defined as a history of laser photocoagulation treatment.

5.5 Definition of coronary heart disease, heart failure, and stroke

In Study IV, follow-up data for incident CHD, heart failure and stroke events were based on the Finnish Care Register for Health Care or the Cause of Death Register. The following ICD and other codes were used for CHD: myocardial infarction ICD-8/9: 410 (www.icd9data.com/2007/Volume1) or ICD-10: I21–22 (http://apps.who.int/classify-cations/icd10/browse/2016/en) or coronary intervention codes for coronary artery bypass surgery or balloon angioplasty, procedure codes based on the Nordic Medico-Statistical Committee: TFN40, FN1AT, FN1BT, FN1YT, FNF, FNG, FNA, FNB, FNC, FND and FNE (since 1996 http://nordclass.se/ncsp_e.htm) and surgical procedure codes according to the procedure classification of the Finnish Hospital Association 1983–1995: 5311– 5315 (348); heart failure ICD-8: 4270, 4271, 7824, ICD-9: 4280–4289, ICD-10: I50; and ischemic/ hemorrhagic stroke ICD-8/9: 430–434, ICD-10: I60–I64.

5.6 Assessment of lifestyle factors

5.6.1 Alcohol consumption

At the baseline visit, all participants were asked to fill in questionnaires regarding their alcohol consumption. Participants reported their weekly consumption of different beverage types, namely beer (one third of a liter bottles), wine (glasses), and stronger spirits (deciliters). The amount of alcohol consumed was first transformed into standard drinks that contain 12 g of pure alcohol. The equivalent dose for one standard drink is 33 cl beer, 12 cl wine, and 4 cl spirits. The total alcohol consumption in g per week was then calculated. In Study I, the participants were grouped in five different groups based on their weekly alcohol consumption—abstainers, light consumers, moderate consumers, heavy consumers, and former consumers, who no longer were consuming alcohol.

There is no international consensus regarding the different levels of alcohol consumption, and therefore the limits were based on the Finnish Current Care guidelines from 2011 (www.kaypahoito.fi). The limit of heavy drinking was ≥7 doses (84 g) per single time or ≥ 24 doses (288 g) per week for men and ≥5 doses (60 g) per single time or ≥16 doses (192 g) per week for women. The limit for a light consumer was

defined as <7 doses per week for men and <5 doses per week for women. Moderate consumers were consuming more than light drinkers but less than the weekly heavy drinking limits.

In Study I, participants were also grouped based on the beverage type they were mainly consuming. If a participant were consuming one type of beverage ≥75% of the total consumption in g/week, they were considered wine, beer, or spirit consumers; otherwise they were considered mixed consumers.

5.6.2   Smoking

During the baseline visit, participants were asked to fill in questionnaires regarding their current and former smoking habits. Based on the FinnDiane protocol, the participants were considered smokers if they had smoked at least one cigarette per day for at least one year. Otherwise, they were considered never smokers. Participants who had stopped smoking before their baseline visit were considered former smokers.

Data regarding the participants' smoking habits were also assessed during the prospective visits and through a mailed questionnaire in 2015. Additional smoking data from prospective visits were available for 1566 participants and data from the follow-up questionnaire were available for nearly 2000 individuals. Based on these data, the baseline smoking status was corrected for 46 participants and missing smoking status was reconstructed for 118 participants. These additional smoking data were available in Study IV.

The percentages of current smokers at the baseline visits and in the 2015 follow-up questionnaire are shown in **Figure 2**. During the last 20 years, the proportion of men who were current smokers at the baseline visit declined from 30.6% in 1994–1998 to 19.7% in 2010–2014. For women, the percentage of current smokers started to decline only during the last 10 years from 24.4% in 2003–2004 to 17.0% in 2010–2014. Based on the follow-up questionnaire sent to the participants in 2015, only 9.9% of men and 9.3% of women were current smokers. However, the participants who answered the questionnaire were older (median age 52.5) than the participants who enrolled in the FinnDiane study during the years 2010–2014 (median age 37.9).
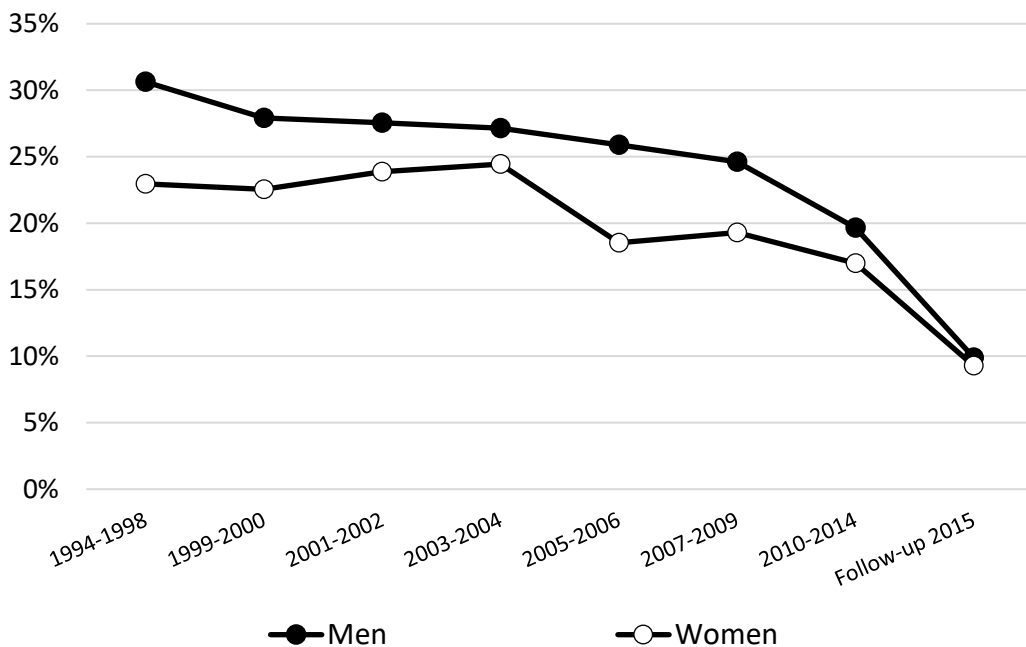
Figure 2. Percentage of current smokers at the baseline visits and according to the follow-up questionnaire in 2015

Questionnaires also included questions about the year a participant started smoking and the year of smoking cessation if a participant was a former smoker or had several different time periods of smoking. Participants were also asked to report the number of cigarettes per day they smoked during different time periods.

Based on this information, the cumulative smoking in pack-years was calculated for each participant. By definition, smoking 20 cigarettes per day in a period of one year, equals one pack-year. The pack-year data were used in Study II and Study IV. Study IV also used the intensity of smoking as cigarettes per day and packs (20 cigarettes) per day as a measurement for the dosage of smoking.
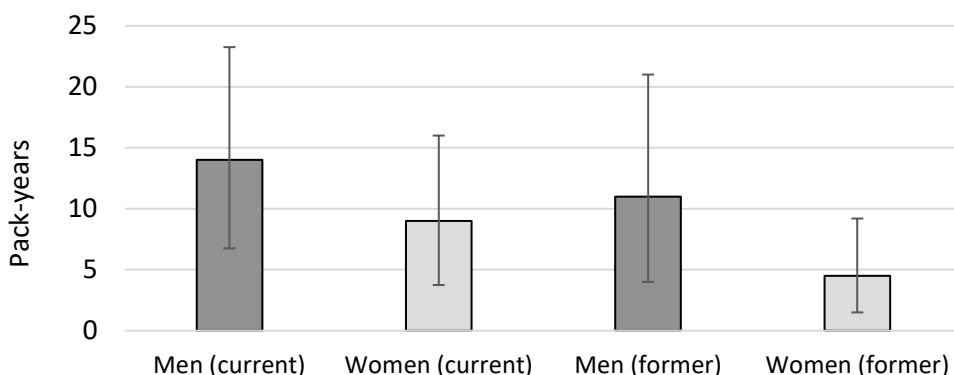
Figure 3. Cumulative smoking in pack-years (median, IQR) in current and former smokers at baseline visit

Figure 3 shows the average cumulative smoking in pack-years for current and former smokers at baseline. Men who were current smokers had smoked 14 pack-years and women who were current smokers had smoked 9 pack-years. Among former smokers, the corresponding numbers were 11 for men and 4.5 for women. **Figure 4** shows the average intensity of smoking in cigarettes per day in current and former smokers at baseline. Among current smokers, men were smoking 15 cigarettes per day and women 10. In former smokers, the intensity of smoking was 18 cigarettes per day in men and 10 in women. The median age when the participants started smoking was 17 (interquartile range [IQR]) 15–20), which was the same for men and women and also for current and former smokers. The median age of smoking cessation was 33 (IQR 26–41) for men and 28 (IQR 23–36) for women.
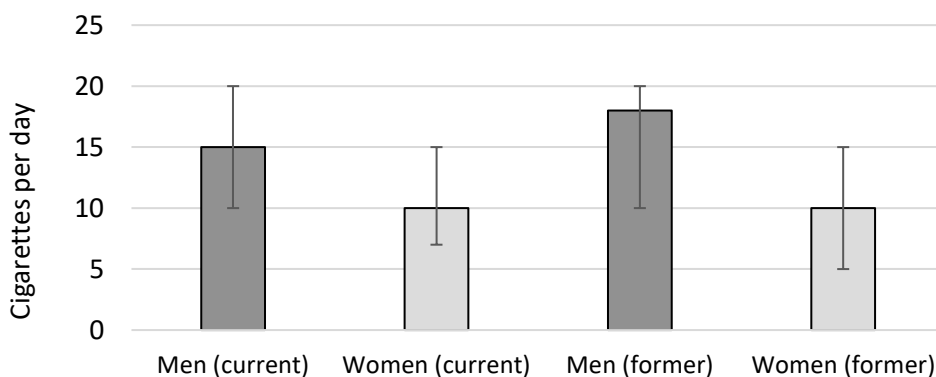
Figure 4. Intensity of smoking in cigarettes per day (median, IQR) in current and former smokers at baseline visit

5.7 Genotyping

For Study III, the genetic variant rs4972593 was extracted from an existing GWAS. The genotyping was performed with the Illumina 610Quad chip, and the quality control and genotype imputation was based on the HapMapII CEU population. The imputed genotype probabilities of rs4972593 were converted to the most likely genotypes using a probability threshold of 0.9 for genotype calling. After the conversion, the genotype call rate was 0.98, the minor A allele frequency was 0.11, and no deviation from the Hardy–Weinberg disequilibrium was observed (p=0.72). The imputation quality of rs4972593 was good (MACH: quality=0.99, Rsq=0.95). For Study III, data for the genotype, smoking history, and ESRD were available for 2621 patients.

5.8 Statistical analyses

In Study I, Study II, and Study IV, data regarding baseline characteristics are presented as means ± standard deviation for normally distributed values and otherwise as medians (IQR). Categorical variables are reported as percentages. Differences between groups were analyzed using ANOVA for normally distributed continuous variables and otherwise using the Kruskal–Wallis test. Differences between categorical variables were analyzed using the χ^2 test. In all studies, the statistical analyses were performed with IBM SPSS statistics versions 22–24 (IBM Corporation, Armonk, NY, USA) and SAS versions 9.2–9.4 (SAS Institute, Cary, NC, USA).

5.8.1 Study I

The cross-sectional associations between alcohol consumption or beverage type and diabetic nephropathy or severe diabetic retinopathy were calculated with logistic regression analyses, providing odds ratios (OR) with 95% confidence intervals (CI). The covariates included in the multivariable models were age at onset of diabetes, sex, duration of diabetes, triglycerides, HDL cholesterol, HbA$_{1c}$, social class, BMI, smoking status, hypertension, and lipid-lowering medication. In the second model eGDR was included and HbA$_{1c}$, BMI, and hypertension were excluded. The interaction term between sex and amount of alcohol consumed or beverage type was entered into the models, and if the interaction term was significant the analyses were performed

separately for men and women. Light consumers and wine drinkers were used as reference categories in the analyses.

5.8.2 Study II

The 12-year cumulative incidence of micro- and macroalbuminuria among the participants with different baseline smoking status was estimated using the Kaplan–Meier method. A log-rank test was used to test the differences between the groups. Fine and Gray´s test with death as the competing risk was used when analyzing the 12-year cumulative risk of ESRD. Cox regression models were used for the analyses regarding cumulative smoking, measured with pack-years as the continuous variable, providing hazard ratios (HRs) with a 95% CI for the development of different stages of diabetic nephropathy. Cox regression models were also used in analysing the combined risk of any progression of diabetic nephropathy and the risk of ESRD associated with baseline smoking status. The variables included in the stepwise models were sex, duration of diabetes, HbA_{1c}, SBP, HDL cholesterol and triglycerides, BMI, and social class. In former smokers, the effect of smoking cessation was analyzed using the years between quitting and the baseline visit as a continuous variable in the Cox regression models. Cubic spline graphs were used to estimate the association between continuous pack-years and the risk of any diabetic nephropathy and ESRD.

5.8.3 Study III

The combined effect of history of smoking (never vs. ever) and the rs4972593 variant on the 40-year cumulative risk of ESRD was estimated using the Kaplan–Meier method. A log-rank test was used to test the differences between the groups. Cox regression models were also used, providing HRs for the risk of ESRD. The third-degree interaction term between sex, smoking, and the minor allele was significant with respect to the development of ESRD (p=0.001), indicating that the effect of the rs4972593 on the progression of ESRD is dependent on smoking status and is different for men and women. Therefore, the analyses were conducted separately for men and women.

5.8.4 Study IV

In Study IV, the effect of smoking status, cumulative smoking in pack-years, and smoking intensity in packs per day were analyzed using Cox regression models, providing HRs with 95% CI for the incidence of CHD, heart failure and stroke. Possible confounding factors were included in the models in different steps and combinations. These included environmental risk factors (social class and alcohol intake) and traditional CVD risk factors (age, sex, HbA_{1c}, hypertension, duration of diabetes, BMI, HDL cholesterol, triglycerides, and baseline presence of diabetic nephropathy). The interaction term between sex and smoking status, intensity of smoking, and cumulative smoking was entered into the models, and if the interaction term was significant the analyses were performed separately for men and women. Finally, the results regarding the risk of CHD and stroke were combined with those of previous studies in a small meta-analysis conducted using random-effects models by the %METAANAL SAS macro.

6   RESULTS

6.1   Alcohol consumption and the risk of diabetic nephropathy and severe diabetic retinopathy (Study I)

At baseline, 858 of the 3608 participants were abstainers, 1690 were light consumers of alcohol, 799 were moderate consumers, 120 were heavy consumers, and 141 were former consumers. The proportions of some of these groups differed between men and women (**Figure 5**). The percentage of abstainers was higher (31.8%) among women compared to men (16.5%, p <0.0001). The proportion of light consumers was similar among men (47.0%) and women (46.6%). Percentages of moderate consumers (27.5% vs. 16.2% p <0.0001) and heavy consumers (5.1% vs. 1.4%, p <0.0001) were higher among men compared with women. The proportion of former consumers did not differ between genders (3.9% in both).
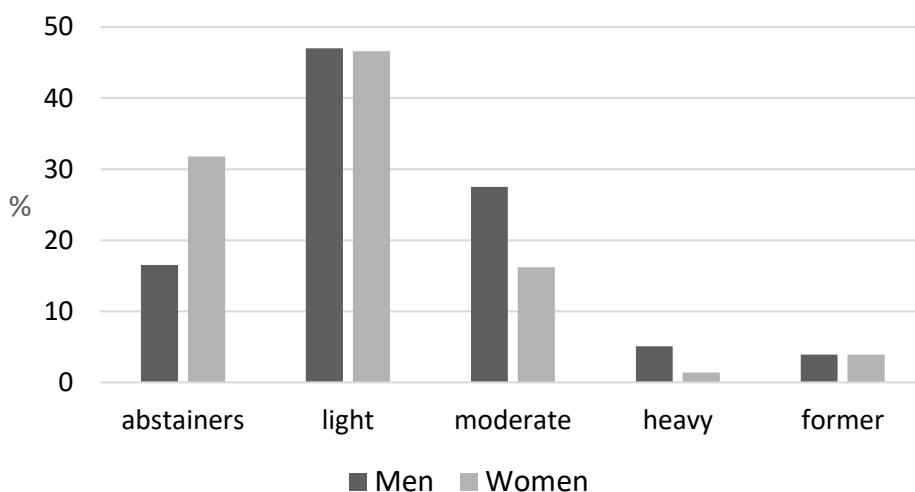
Figure 5. Proportion of abstainers and light, moderate, heavy, and former consumers of alcohol at baseline in men and women

Among the participants who were current consumers of alcohol, the number of wine consumers was 322, the number of beer consumers was 1245, the number of spirit consumers was 175, and the number of mixed drinkers was 867. **Figure 6** shows the baseline percentages of participants consuming different beverage types in men and women. There were fewer wine consumers among men (4.6%) compared to women (22.9%, p <0.0001). Otherwise, there were more beer (50.7% vs. 43.8%, $p$ <0.001), spirit (8.4% vs. 4.5%, p <0.0001), and mixed consumers (36.4% vs. 28.9%, p <0.0001) among men compared to women.
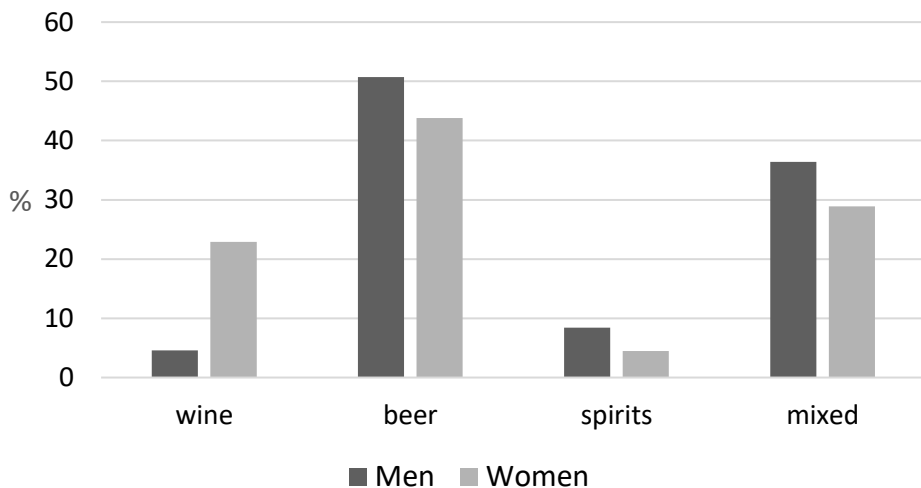
Figure 6. Proportion of wine, beer, spirit, and mixed consumers at baseline in men and women

Baseline characteristics differed between the groups. Heavy consumers were the oldest and had the highest total cholesterol and triglyceride concentrations and the highest blood pressure. HDL cholesterol was the highest among moderate and heavy consumers. Former consumers had the longest duration of diabetes and the poorest glycemic control. Among current alcohol consumers, spirit drinkers were the oldest and had the longest duration of diabetes and the poorest lipid profile.

The prevalence of diabetic nephropathy (i.e. macroalbuminuria or ESRD) at baseline is presented in **Figure 7**. The percentage of participants with diabetic nephropathy was the lowest in light consumers of alcohol (17.6%) and the highest in former consumers (45.4%). In addition, the prevalence of severe diabetic retinopathy was the lowest in light consumers (29.6%) and the highest in former consumers (52.2%) (**Figure 8**). Therefore, light consumers were used as reference category in the logistic regression models.
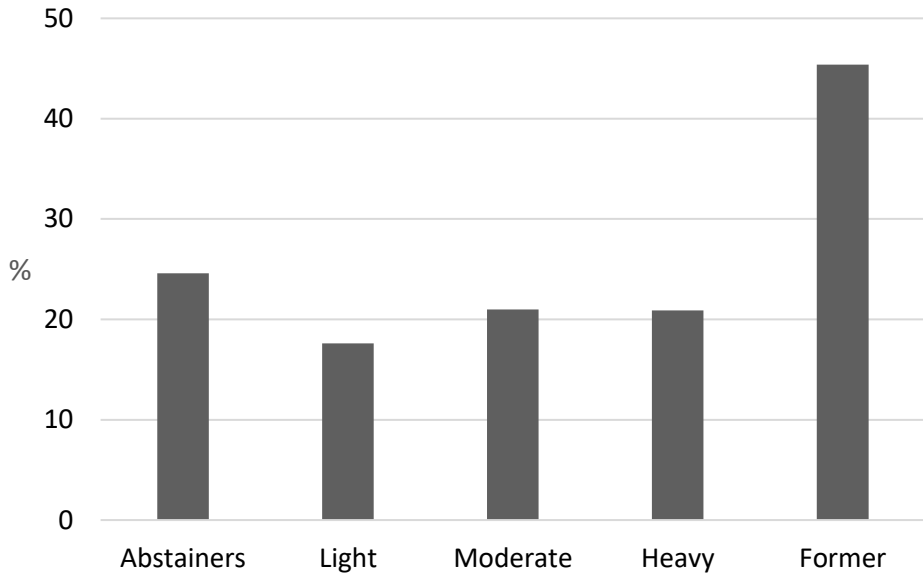
Figure 7. Baseline prevalence of diabetic nephropathy stratified by the amount of alcohol consumed
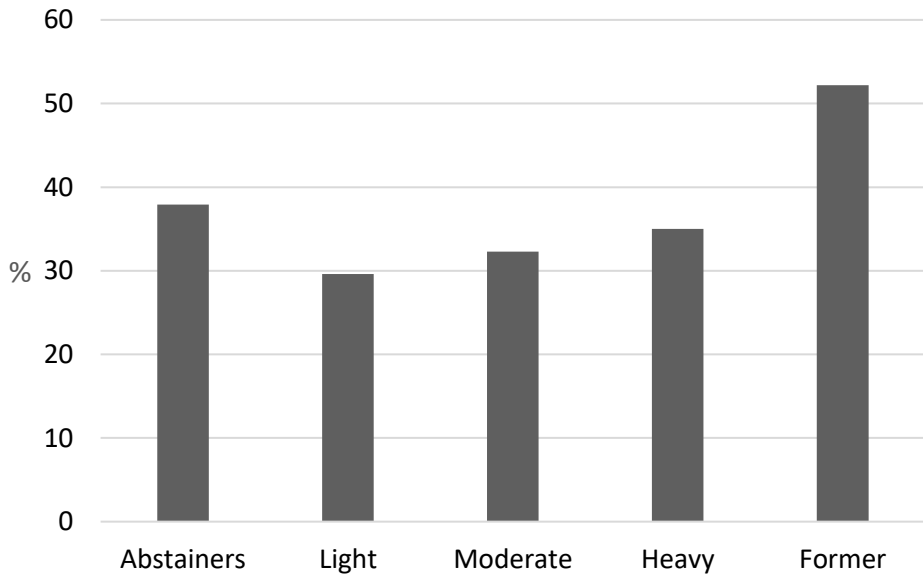
Figure 8. Baseline prevalence of severe diabetic retinopathy based on the amount of alcohol consumed

The prevalence of diabetic nephropathy differed between men and women in different groups based on beverage types. Beer-drinking men had the lowest prevalence of diabetic nephropathy (16.7%), but in women the prevalence of diabetic nephropathy was similar for wine drinkers (12.6%), beer drinkers (13.8%), and mixed drinkers (14.2%). Spirit drinkers had the highest prevalence of diabetic nephropathy in both men (45.0%) and women (23.4%) (**Figure 9**). The prevalence of severe diabetic retinopathy was highest in spirit drinkers (52%) (**Figure 10**).
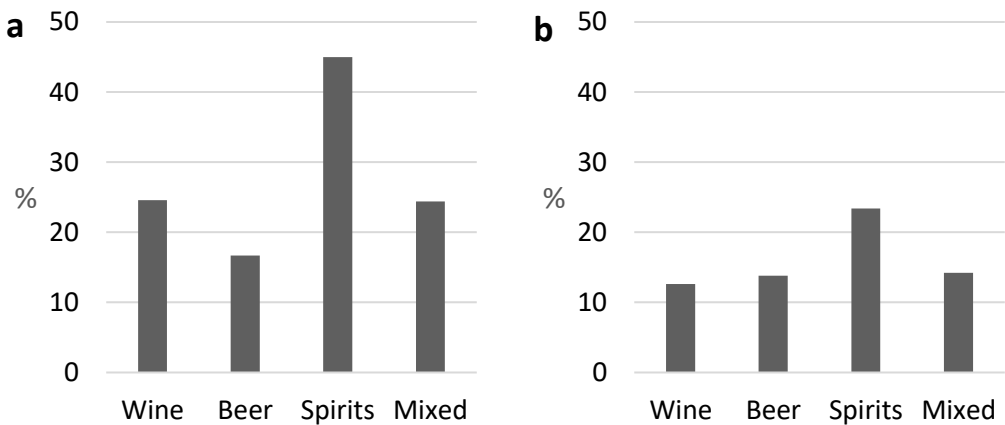
Figure 9. Baseline prevalence of diabetic nephropathy stratified by beverage type in men (a) and women (b)
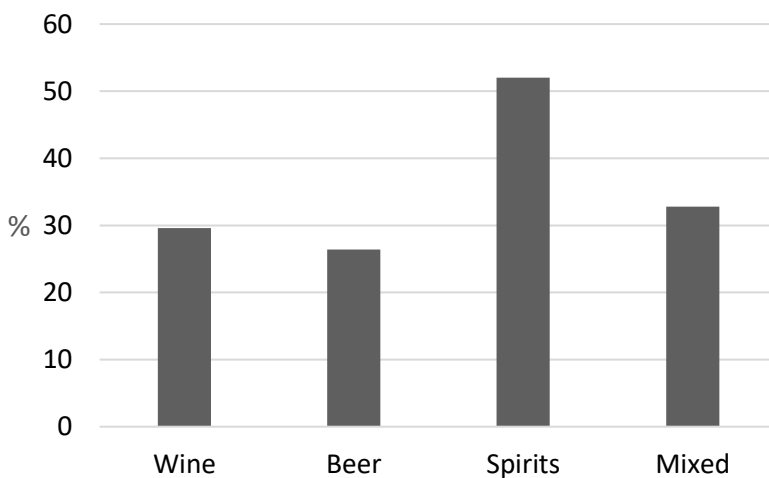
Figure 10. Baseline prevalence of severe diabetic retinopathy based on beverage type

Table 4 presents the results of the logistic regression analyses regarding the association between the amount of alcohol consumed and diabetic nephropathy and severe diabetic retinopathy. Compared with light consumers, former drinkers had the highest risk of diabetic nephropathy with an OR of 2.44 (95% CI 1.49–3.99), adjusted for age at onset of diabetes, sex, duration of diabetes, triglycerides, HDL cholesterol, HbA$_{1c}$, social class, BMI, smoking status, hypertension, and lipid-lowering medication. Abstainers had a higher risk of diabetic nephropathy with an OR of 1.39 (95% CI 1.05–1.84) compared with light consumers. The risk of diabetic nephropathy in moderate and heavy consumers did not differ from that in light consumers. The results regarding the risk of severe diabetic retinopathy were similar, with a higher risk in former drinkers [OR 1.73 (95% CI 1.07–2.79)] and abstainers [OR 1.42 (95% CI 1.11–1.82)] compared with light consumers. In the model including the eGDR, the results for the risk of diabetic nephropathy did not change significantly, and former drinkers [2.18 (95% CI 1.29–3.69)] and abstainers [1.39 (95% CI 1.04–1.87)] still had a higher risk compared with light consumers. However, regarding the risk of severe diabetic retinopathy, the results in former drinkers were attenuated, and when eGDR was included in the model only the abstainers had a significantly higher risk compared with light consumers.

Table 4. Odds ratios for the risk of diabetic nephropathy and severe diabetic retinopathy according to alcohol consumption

	n	cases	OR1 (95% CI)	*P* value	OR2 (95% CI)	*P* value
Nephropathy						
Light consumers	1605	283	Reference		Reference	
Moderate consumers	749	157	0.97 (0.73–1.29)	0.822	0.87 (0.65–1.18)	0.378
Heavy consumers	110	23	0.77 (0.41–1.44)	0.404	0.58 (0.31–1.10)	0.093
Former drinkers	130	59	2.44 (1.49–3.99)	<0.001	2.18 (1.29–3.69)	<0.01
Abstainers	811	199	1.39 (1.05–1.84)	<0.05	1.39 (1.04–1.87)	<0.05
Severe retinopathy						
Light consumers	1686	497	Reference		Reference	
Moderate consumers	797	257	0.94 (0.74–1.21)	0.648	0.91 (0.71–1.18)	0.486
Heavy consumers	120	42	0.91 (0.53–1.55)	0.715	0.84 (0.49–1.44)	0.527
Former drinkers	137	71	1.73 (1.07–2.79)	<0.05	1.51 (0.92–2.49)	0.102
Abstainers	854	323	1.42 (1.11–1.82)	<0.01	1.43 (1.11–1.84)	<0.01

OR1: Adjusted for age at onset, sex, smoking, duration of diabetes, triglycerides, HDL cholesterol, social class, HbA$_{1c}$, hypertension, BMI, and lipid-lowering medication

OR2: Adjusted for age at onset, sex, smoking, duration of diabetes, triglycerides, HDL cholesterol, social class, lipid-lowering medication, and estimated glucose disposal rate (eGDR)

Table 5 presents the results of the logistic regression analyses regarding the association between the beverage type and diabetic nephropathy and severe diabetic retinopathy. The interaction term between sex and beverage type was significant for the risk of diabetic nephropathy; therefore, men and women were analyzed separately. Compared with wine consumers, spirit-drinking men had a higher risk of diabetic nephropathy with an OR of 2.80 (95% CI 1.15–6.81). In women, no significant difference was found between those consuming different types of beverages regarding the risk of diabetic nephropathy. Regarding the risk of severe diabetic retinopathy, there was no interaction between sex and type of beverage. Therefore, men and women were pooled for the analysis. Spirit consumers had a higher risk of severe diabetic retinopathy with an OR of 2.32 (95% CI 1.35–4.00) compared with wine consumers. Regarding the risk of severe diabetic retinopathy, no difference between wine and beer consumers or mixed consumers was observed. When eGDR was entered into the models, the risk of diabetic nephropathy in spirit-drinking men and the risk of severe diabetic retinopathy in all spirit consumers was no longer significantly higher compared with wine consumers.

Table 5. Odds ratios for the risk of diabetic nephropathy and severe diabetic retinopathy according to beverage type

	n	cases	OR1 (95% CI)	*P* value	OR2 (95% CI)	*P* value
Nephropathy men						
Wine	65	16	Reference		Reference	
Beer	714	119	0.83 (0.37–1.82)	0.632	0.71 (0.32–1.59)	0.407
Spirits	120	54	2.80 (1.15–6.81)	0.023	2.34 (0.96–5.71)	0.062
Mixed	521	127	1.33 (0.60–2.92)	0.481	1.09 (0.50–2.38)	0.834
Nephropathy women						
Wine	238	30	Reference		Reference	
Beer	464	64	1.22 (0.65–2.31)	0.536	1.12 (0.59–2.12)	0.730
Spirits	47	11	0.84 (0.26–2.69)	0.766	0.64 (0.21–2.03)	0.455
Mixed	296	42	0.96 (0.48–1.91)	0.897	0.80 (0.40–1.59)	0.527
Severe retinopathy						
Wine	321	95	Reference		Reference	
Beer	1241	327	1.26 (0.86–1.84)	0.246	1.09 (0.74–1.61)	0.664
Spirits	176	90	2.32 (1.35–4.00)	0.002	1.69 (0.97–2.94)	0.063
Mixed	866	284	1.32 (0.89–1.97)	0.166	1.08 (0.73–1.60)	0.712

OR1: Adjusted for age at onset, sex, smoking, duration of diabetes, triglycerides, HDL cholesterol, social class, HbA_{1c}, hypertension, BMI, and lipid-lowering medication

OR2: Adjusted for age at onset, sex, smoking, duration of diabetes, triglycerides, HDL cholesterol, social class, lipid-lowering medication, and estimated glucose disposal rate (eGDR)

6.2 Smoking and the risk of diabetic nephropathy (Study II)

Among the 3613 participants, diabetic nephropathy status progressed in 198 (23.0%) current smokers, 133 (19.6%) former smokers, and 219 (12.4%) never smokers during a median follow-up of 6.8 (IQR 4.3–10.6) years. Altogether, 99 (10.6%) current smokers, 79 (10.7%) former smokers, and 115 (5.9%) never smokers developed ESRD during a follow-up of 12.2 (IQR 10.0–14.0) years. Current smokers had the poorest glucose control and were more insulin-resistant compared with the others. Former smokers were the oldest and had the longest duration of diabetes, and never smokers had the most favorable lipid profile.

Table 6. Risk of any progression of diabetic nephropathy and ESRD according to baseline smoking status

Smoking status	n (%)	events	HR1 (95% CI)	P value	HR2 (95% CI)	P value	HR3 (95% CI)	P value
Progression								
Never	1766	219	Reference		Reference		Reference	
Current	861	198	1.68 (1.36–2.07)	<0.0001	1.54 (1.24–1.92)	<0.0001	1.46 (1.17–1.83)	<0.001
Former	677	133	1.29 (1.02–1.62)	0.034	1.23 (0.97–1.57)	0.093	1.21 (0.95–1.55)	0.131
ESRD								
Never	1946	115	Reference		Reference		Reference	
Current	932	99	1.68 (1.26–2.25)	<0.001	1.47 (1.09–1.99)	0.013	1.42 (1.04–1.95)	0.027
Former	735	79	1.35 (0.99–1.84)	0.055	1.23 (0.89–1.70)	0.217	1.24 (0.88–1.73)	0.215

HR1: Adjusted for sex, duration of diabetes, HbA_{1c}, and systolic blood pressure
HR2: Adjusted for sex, duration of diabetes, HbA_{1c}, systolic blood pressure, HDL cholesterol, triglycerides, and BMI
HR3: adjusted for sex, duration of diabetes, HbA_{1c}, systolic blood pressure, HDL cholesterol, triglycerides, BMI, and social class

Table 6 presents the Cox regression models for the progression of diabetic nephropathy and the development of ESRD. Current smokers had a higher risk of any progression of diabetic nephropathy with a HR of 1.46 (95% CI 1.17–1.83) compared with never smokers after adjustments for sex, duration of diabetes, HbA_{1c}, SBP, HDL cholesterol, triglycerides, BMI, and social class. The risk of ESRD was higher in current smokers with a HR of 1.42 (95% CI 1.04–1.95) compared with never smokers. The risk of any

progression or of ESRD in former smokers compared with never smokers did not differ significantly after adjustments for other covariates.

When the association between the cumulative smoking dose in pack-years and the risk of different stages of diabetic nephropathy were assessed, current smokers had a higher risk of macroalbuminuria with a HR of 1.025 (95% CI 1.010–1.041) per each pack-year and a higher risk of ESRD with a HR of 1.041 (95% CI 1.001–1.026) compared with never smokers. Former smokers had a higher risk of microalbuminuria with a HR of 1.034 (95% CI 1.014–1.054) per pack-year compared with never smokers, but the risk of macroalbuminuria and ESRD was similar.

In former smokers, the risk of macroalbuminuria decreased by 7% per each year without smoking before the baseline visit. When former smokers were grouped in 5-year intervals based on the time since quitting before baseline, the risk was similar in former smokers compared with current smokers when they quit smoking less than 5 years before baseline. However, with increasing years since smoking cessation, the risk approached the risk seen in never smokers (as shown in **Figure 11)** based on Cox regression models adjusted for sex, duration of diabetes, and HbA$_{1c}$.
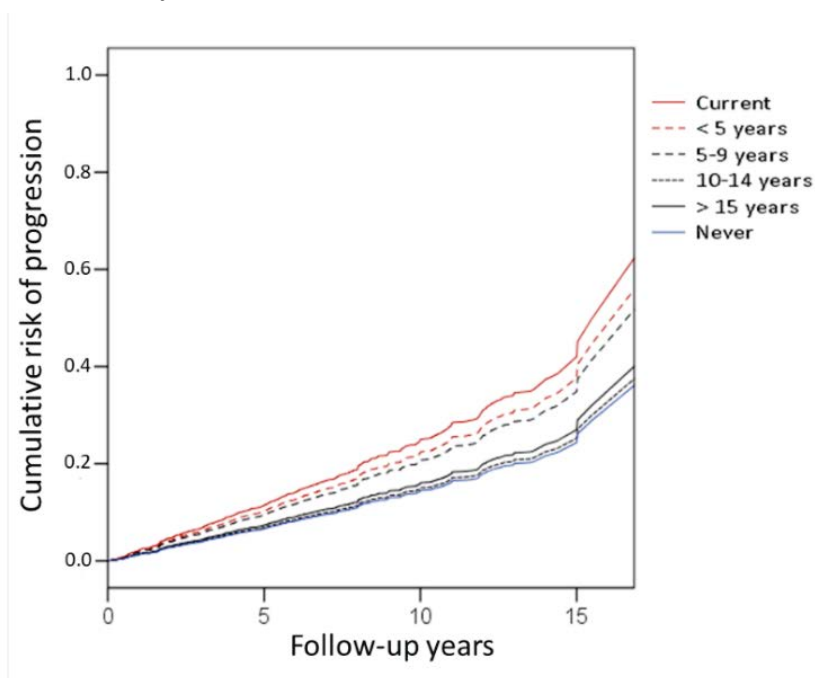
Figure 11. Cumulative risk of any progression of diabetic nephropathy during follow-up based on smoking status and years since smoking cessation before baseline in former smokers

6.3 The combined effect of smoking and the rs4972593 allele on the development of end-stage renal disease (Study III)

The follow-up of Study III ended in 2013, and by then 36 (24.7%) non-smoking women with the rare allele rs4972593 had developed ESRD compared with 66 (10.8%) non-smoking women without the rare allele (**Table 7a**). In men, the results were the opposite; by 2013, 14 (11.8%) non-smoking men with the rare allele had developed ESRD compared with 104 (21.4%) non-smoking men without the rare allele (**Table 7b**). In women, the time period from diagnosis of diabetes to ESRD was longer, and the age at ESRD diagnosis was higher in non-smokers without the rare allele compared to non-smokers with the rare allele (**Table 7a**). Again, in men the findings were the opposite, and the time period to ESRD was longer and the age at ESRD diagnosis was higher in non-smokers with the rare allele (**Table 7b**). Among smokers, there were no differences between the groups.

Table 7a. Participant characteristics according to smoking status and rs4972593 allele for women

	Smokers		Non-smokers		P value (smokers)	P value (non-smokers)
	with rare allele	without rare allele	with rare allele	without rare allele		
n	121	428	146	609		
ESRD 2013 (%)	26.4	19.6	24.7	10.8	0.105	<0.0001
Time period to ESRD (years)	34 (28–42)	34 (28–43)	35 (28–43)	37 (31–45)	0.871	0.009
Age at the ESRD diagnosis (years)	49 (40–57)	49 (41–58)	47 (38–56)	51 (43–60)	0.519	0.002

Table 7b. Participant characteristics according to the smoking status and rs4972593 allele for men

	Smokers		Non-smokers		P value (smokers)	P value (non-smokers)
	with rare allele	without rare allele	with rare allele	without rare allele		
n	148	565	119	485		
ESRD 2013 (%)	33.8	28.1	11.8	21.4	0.179	0.017
Time period to ESRD (years)	36 (30–42)	35 (28–42)	37 (31–45)	35 (28–42)	0.510	0.005
Age at the ESRD diagnosis (years)	50 (42–60)	52 (42–60)	52 (45–59)	49 (40–58)	0.677	0.031

Values are presented as median (IQR) or percentages. *P*-values were obtained using a Kruskal–Wallis test or a χ^2 test.

The 40-year cumulative risk of ESRD was 27.1% (95% CI 20.6–33.1) in non-smoking women with the minor allele compared with 10.5% (95% CI 7.9–13.0) in non-smoking women without the minor allele (p <0.0001, HR 2.62 [95% CI 1.74–3.93]). In women who were ever smokers, the minor allele carriers had around a 10% higher 40-year cumulative risk of ESRD [32.9% (95% CI 25.6–40.0)], but the difference was not significant compared with women without the minor allele [23.4% (95% CI 19.4–27.1)] (p=0.117).

In non-smoking men, the effect of the minor allele was the opposite. In non-smoking men with the minor allele, the 40-year cumulative risk of ESRD was 12.3% (95% CI 5.9–18.4) compared to 24.6% (95% CI 20.9–28.1) in non-smoking men without the minor allele (p=0.007, HR 0.47 [95% CI 0.27–0.82]). In men who were ever smokers, the minor allele did not alter the cumulative risk of ESRD. Smoking men with the minor allele had a 31.8% (95% CI 25.5–37.6) cumulative risk of ESRD compared with a 32.0% cumulative risk (95% CI 28.8–35.0) (p=0.336) in smoking men without the minor allele.

6.4 Smoking and the risk of coronary heart disease, heart failure, and stroke (Study IV)

Among the 4506 participants, 130 (11.6%) current smokers, 139 (14.8%) former smokers, and 243 (10.7%) never smokers developed CHD during the follow-up. The respective numbers for heart failure were 72 (6.3%), 110 (10.9%), and 131 (5.7%). For any stroke in men, the respective numbers were 63 (9.8%), 75 (13.2%), and 53 (5.0%), and for any stroke in women the respective numbers were 27 (5.6%), 20 (5.0%), and 66 (5.3%).

Figure 12 presents the age and sex-adjusted cumulative risk of CVD based on baseline smoking status. Current smokers had a higher risk of CHD with a HR of 1.31 (95% CI 1.05–1.63), but in former smokers the risk of CHD was similar to that in never smokers, with a HR of 1.10 (95% CI 0.89–1.36) (**Figure 12a**). In contrast, the risk of heart failure was higher in current smokers with a HR of 1.43 (95% CI 1.07–1.92) and in former smokers with a HR of 1.52 (95% CI 1.17–1.96) (**Figure 12b**). In men, the risk of any stroke was also higher in current smokers with a HR of 2.20 (95% CI 1.52–3.17) and in former smokers with a HR of 2.06 (95% CI 1.45–2.95) compared with never smokers (**Figure 12c**). However, in women the risk of any stroke did not differ between the groups; for current smokers the HR was 1.32 (95% CI 0.84–2.08), and for former smokersit was 0.89 (95% CI 0.54–1.45) (**Figure 12d**).
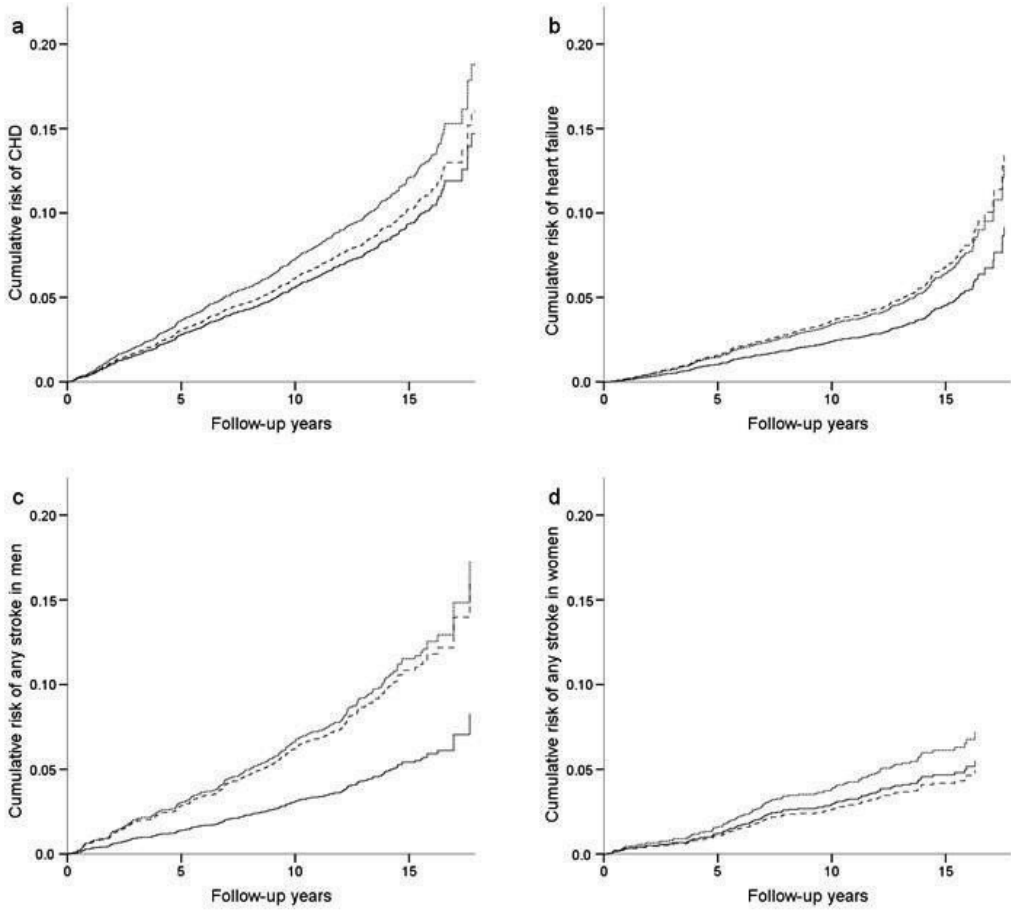
Figure 12. Age and sex adjusted cumulative risk of coronary heart disease (CHD) (a), heart failure (b), any stroke in men (c) and any stroke in women (d) during the follow-up. Solid line = never smokers, dashed line = former smokers and dotted line = current smokers

After further adjustments for other cardiovascular risk factors, the risk of CHD in current smokers, and the risk of heart failure in current and former smokers was attenuated, and after the lipid variables were added into the model, the difference compared with never smokers was no longer statistically significant. However, in men who were current or former smokers the risk of any stroke remained significantly higher, as it did after adjustments for other CVD risk factors, including lipids. Notably, in men the risk of stroke was increased even after diabetic nephropathy was included in the model, with a HR of 1.90 (95% CI 1.29–2.82) for current smokers and of 1.92 (95% CI 1.32–2.80) for former smokers.

When the effect of intensity of smoking in packs per day on the risk of different CVD outcomes was assessed, the risk of CHD increased, with a HR of 1.28 (95% CI 1.00–1.63) in current smokers per one pack per day. The risk of heart failure was also higher in current and former smokers compared with never smokers, but after the results were adjusted for the lipid variables the difference was no longer statistically significant. The risk of any stroke increased with a HR of 1.50 (95% CI 1.09–2.05) with intensity of one pack per day in current smokers and a HR of 1.45 (95% CI 1.10–1.90) in former smokers compared with never smokers.

When addressing the CVD risk associated with cumulative smoking in pack-years, the risk of CHD in current smokers and heart failure in current and former smokers increased by 1–1.5% per each pack-year. Again, the effect of cumulative smoking was stronger on the risk of stroke, which increased by 1.5–2% per one pack-year in both current and former smokers.

In a small meta-analysis, the results from Study IV were combined with the results of three other studies regarding the risk of CHD and one other study regarding the risk of stroke. Based on the results, the risk of CHD was higher in current smokers with a risk ratio (RR) of 1.36 (95% CI 1.07–1.72), and the risk of stroke was increased in men with a RR of 1.77 (95% CI 1.00–3.14) in current smokers and with a RR of 2.13 (95% CI 1.52–3.00) in former smokers.

7 DISCUSSION

7.1 Effect of alcohol consumption and beverage type on the risk of diabetic nephropathy and severe diabetic retinopathy

Study I showed that in former alcohol consumers the risk of diabetic nephropathy was doubled, and the risk of severe diabetic retinopathy was 50–70% higher compared with light consumers. In abstainers, the risk of diabetic nephropathy and severe diabetic retinopathy was around 40% higher compared with light consumers. However, the risk of diabetic nephropathy or severe diabetic retinopathy was not increased in current moderate or heavy consumers compared with light consumers. When the comparison was made between different beverage types, a higher risk of diabetic nephropathy was seen only in men who were spirit drinkers, but the risk of severe diabetic retinopathy was higher in all spirit drinkers.

The EURODIAB study showed an association between moderate alcohol consumption and a decreased risk of microvascular complications in people with type 1 diabetes (332). The study used abstainers as a reference group and only observed a trend towards a lower risk in moderate consumers. However, the actual risk of different microvascular outcomes was not significantly higher in heavier consumers compared with participants with lower alcohol consumption. Thus, the results are in line with those of Study I. An older cross-sectional report from the WESDR showed an inverse association between alcohol consumption and the prevalence of proliferative diabetic retinopathy (334). However, a later WESDR prospective study did not find any association between alcohol consumption and the incidence or progression of diabetic retinopathy (335). It is of note that in the prospective WESDR study, alcohol consumption was analyzed as a continuous variable. This approach does not necessarily provide information on different alcohol consumption levels, considering the previously known non-linear association between alcohol consumption and vascular complications. In the DCCT/EDIC study, occasional or regular drinking was not associated with a higher risk of proliferative diabetic retinopathy compared with abstaining (168). In addition, in Study I the risk of diabetic nephropathy or severe diabetic retinopathy seems to stay at a similar level regardless of the amount of alcohol consumed. A similar L-shaped curve is also seen in studies regarding alcohol consumption and the risk of CHD in people with type 2 diabetes (327). However, in the general population, this L-shaped association between alcohol consumption and CHD is only seen in men (324). In Study I, men and women were pooled because there was

no significant interaction between sex and the amount of alcohol consumed and the risk of diabetic nephropathy or severe diabetic retinopathy. However, the number of women who were heavy consumers was clearly lower compared to men (24 or 1.4% vs. 96 or 5.1%), and therefore the risk of diabetic nephropathy and severe diabetic retinopathy in heavy consumers refers mostly to the risk seen in men.

In Study I, former consumers of alcohol carried the highest risk of diabetic nephropathy and severe diabetic retinopathy. In the WESDR study, former drinkers had a higher prevalence of proliferative diabetic retinopathy compared with current drinkers, which is in line with results of Study I. Other studies regarding people with type 1 diabetes, including the EURODIAB and the prospective WESDR study, did not report results for former drinkers separately. In these studies, the former drinkers were combined with the life-long abstainers, probably leading to a higher risk seen in abstainers. Therefore, the results of Study I are not directly comparable with those of most of the previous microvascular studies. However, the results are in line with the previous CHD studies that have reported a higher CHD risk in former drinkers (323, 325). It is important to separate the former drinkers from the abstainers to avoid the "sick quitter effect". In addition, in Study I the former consumers of alcohol had the poorest glycemic control and were more insulin-resistant with the highest insulin dose, and together with the heavy consumers they also had the lowest eGDR level. Former consumers were more often taking anti-hypertensive or lipid-lowering medication, and they also had the highest percentage of prevalent CVD. Therefore, it is likely that not only the previous alcohol consumption but the overall impaired health status in former consumers has a detrimental effect on the risk of microvascular complications.

The risk of diabetic nephropathy and severe diabetic retinopathy was also addressed in the individuals drinking different alcoholic beverages. Regarding the risk of diabetic nephropathy, men and women were analyzed separately due to the interaction between sex and beverage type. Compared with wine consumers, men who were consuming mostly spirits had the highest risk of diabetic nephropathy. In women, there were no differences in the risk of diabetic nephropathy between the different beverage types. However, this might be explained by the lower number of spirit-drinking women (49, 4.5%) compared to spirit-drinking men (127, 8.4%) among all alcohol consumers. Regarding the risk of severe diabetic retinopathy, there was no significant interaction between sex and beverage type, and women and men were analyzed together. Again, spirit drinkers had a higher risk of severe diabetic retinopathy compared with wine drinkers. No significant difference in the risk of diabetic nephropathy or severe diabetic retinopathy or between wine and beer or mixed drinking was observed.

To our knowledge, this was the first study to compare the risk of diabetic nephropathy or severe diabetic retinopathy between consumers of different beverage types. The EURODIAB study did not compare the risk of microvascular complications between consumers of different beverage types but only within a specific beverage type; therefore, the results of Study I are not directly comparable with their results. However, they showed an association between moderate wine consumption and a reduced risk of diabetic nephropathy and severe diabetic retinopathy. In addition, moderate beer consumption was associated with a lower risk of diabetic nephropathy. In the EURODIAB study, spirit consumption was not associated with a significant change in the risk of diabetic nephropathy or severe diabetic retinopathy. The results of Study I are in line with a large meta-analysis regarding CVD morbidity and mortality (340). Based on those results, both wine and beer consumers seemed to have some protective effect against CVD, but this effect was not seen in the spirit consumers.

7.2 Mechanisms behind the effect of alcohol consumption and beverage type on microvascular complications

Alcohol consumption has known effects on vascular risk factors, such as blood pressure, lipids, hemostasis, and inflammation. In Study I, SBP and the HDL level increased with the increasing amount of alcohol consumed. Markers for hemostasis were not available, but a marker for inflammation, CRP, was lowest among light and moderate consumers. In people with diabetes, alcohol also affects glucose metabolism and insulin sensitivity. In Study I, wine consumers had the highest eGDR levels and spirit drinkers the lowest. When the results for different beverage types were adjusted for insulin sensitivity, expressed as eGDR, the difference between wine and spirit drinkers was no longer significant. There is some evidence that moderate alcohol consumption, especially consumption of red wine, increases insulin sensitivity (313, 318, 349). Therefore, the higher risk of diabetic nephropathy in men and risk of severe diabetic retinopathy in all individuals who were spirit drinkers might partly be explained by the differences in insulin sensitivity between wine and spirit drinkers.

The lower risk seen in the wine drinkers compared with the spirit drinkers may also originate from the differences in socioeconomic status, diet, or other behavioral risk factors, such as physical exercise, between the groups. However, the results for different beverage types were also significant after adjustments for social class, which accounts for at least part of the socioeconomic differences between the groups.

Not only the beverage type but also the drinking pattern may explain the higher risk in the spirit consumers compared with the wine consumers. Unfortunately, the exact information regarding the amount of alcohol the participants consumed per each drinking occasion was not available. However, the percentage of heavy consumers was higher among spirit drinkers (13.1%) compared with wine drinkers (0.3%, only one person). Therefore, it is likely that the percentage of binge drinkers is also higher among spirit drinkers. There are no data regarding the association between drinking pattern and the risk of microvascular complications, but binge and irregular heavy drinking is associated with a higher risk of CHD and stroke (336, 337, 350). Therefore, an unfavorable drinking pattern might explain part of the higher risk of diabetic nephropathy and severe diabetic retinopathy seen in spirit drinkers compared with wine drinkers.

Even though the risk of diabetic nephropathy or severe diabetic retinopathy did not increase with increasing alcohol consumption, people with type 1 diabetes should not be encouraged to increase their alcohol consumption due to the known harmful effects of alcohol on other health aspects, such as the increased risk of many forms of cancer. In people with type 1 diabetes, heavier drinking is also associated with an increased risk of hypoglycemia and impaired hypoglycemia awareness, leading to an additional detrimental or potentially even lethal effect of heavier alcohol consumption.

7.3 Current smoking and risk of diabetic nephropathy

Current smoking was associated with a 40–50% increased risk of any progression of diabetic nephropathy and the development of ESRD. These results are mostly in line with many older diabetic nephropathy studies (166, 241, 287-291). In some of these studies, the risk of the progression of diabetic nephropathy was up to 2–3 times higher in current smokers compared with never smokers. The lower risk increase seen in Study II might be explained by the inclusion in the analyses of a wider range of other confounding factors. It is of note that many later studies have reported conflicting results and have not been able to show a clear association between smoking and the development of diabetic nephropathy (82, 215, 244, 286, 292). In the Danish studies, this might be explained by a large number of smokers among the study participants and the overall smoking (active and passive) prevalence in the population at the time (82, 292). In addition, some studies did not separate former smokers from either current or never smokers (215, 244).

Different than the previous diabetic nephropathy studies, the effect of cumulative smoking in pack-years on the development of different stages of diabetic nephropathy was also analyzed. Compared with never smokers, the risk of macroalbuminuria was increased by 2.5% and the risk of ESRD by 1.4% per each pack-year in current smokers. The risk of microalbuminuria was also higher in current smokers, but the difference was weaker and no longer statistically significant in the final model that included social class as a confounding factor. At baseline, there were fewer current smokers who had normal UAER compared with the never smokers, 64.6% vs. 75.4%. In addition, the percentages of prevalent micro- and macroalbuminuria were higher in the current smokers compared with the never smokers. This might explain the weaker association with the development of microalbuminuria compared with the effect on later stages of diabetic nephropathy. Estimates with continuous pack-year data were also performed, showing that after 20–25 pack-years the risk of any progression of diabetic nephropathy or ESRD is twice as high in current smokers compared with never smokers.

7.4 The combined effect of smoking and the rs4972593 allele on the development of end-stage renal disease

Study III confirmed the previous findings of an increased risk of ESRD in women who are carriers of the rs4972593 rare allele. Ever smoking and the rare allele were both associated with a 2–3-fold increased risk of ESRD in women. Among smoking women, there was no significant difference between the rare and common allele carriers. However, there was a trend towards a higher 40-year cumulative ESRD risk in women who were both smokers and rare allele carriers compared with smokers without the rare allele (32.9% vs. 23.4%).

In the original GWAS, no association between rs4972593 and ESRD was seen in men when non-smokers and smokers were pooled (189). However, based on the findings of Study III, rs4972593 has a protective effect in men that is only seen in non-smokers. Non-smoking men who carry the rare allele had a 50% lower risk of ESRD compared with non-smoking men without the rare allele. No protective effect was found in smoking men who carried the rare allele. Smoking is a strong risk factor for the development of ESRD, and therefore the effect, either positive or negative, of rs4972593 might not be detected in smokers. However, it is also possible that smoking acts directly through epigenetic mechanisms and changes the risk profile of the genotype. This could explain why the protective effect of rs4972593 was not seen in smoking men.

The possible biochemical mechanism behind the association of rs4972593 and the modified risk of ESRD was elucidated in the earlier FinnDiane GWAS study (189). The location of this SNP is in the intergenic region between genes *SP3* and *CDCA7*. Of these genes, only *SP3* is expressed in the kidneys, and therefore the effect of rs4972593 is probably mediated through the *SP3* gene. *SP3* codes the transcription factor Sp3 that binds to estrogen receptor alpha (ERα) and forms a protein complex that regulates gene expression (351, 352). Based on animal models, estradiol action through ERα plays an important role in the development of chronic kidney disease. In female mice, the kidneys express the third largest number of estradiol-regulated genes after the uterus and pituitary gland, and the expression of these genes is regulated by ERα (353). Female ERα knock-out mice develop a form of chronic kidney disease probably due to elevated testosterone levels. However, the male knock-out mice do not develop kidney disease, despite elevated testosterone levels (354). In addition, in people with type 1 diabetes, changes in the testosterone levels are associated with the development of diabetic kidney disease, and an increase in testosterone is observed in men who have developed ESRD (355). The effect of testosterone via the androgen receptor is also mediated through the Sp1/Sp3 transcriptional network. Therefore, a disturbance in this signaling pathway could lead to a decreased effect of testosterone in male kidneys and eventually to a more beneficial outcome. Experimental studies have shown that the majority of estrogen receptors in the male kidneys consist of ERβ, whereas in the female kidneys the majority are of type ERα (356). Therefore, the gender differences seen in Study III could be explained by the more important role of estrogen and ERα in the development of ESRD in women compared with men.

7.5   Current smoking and the risk of coronary heart disease, heart failure, and stroke

Study IV showed a 30% higher risk of CHD in current smokers compared with never smokers. The association was stronger when smoking was assessed as intensity of smoking in packs per day or as cumulative smoking in pack-years instead of the traditional simple smoking status. The risk of heart failure was also higher in current smokers compared with never smokers, but when the lipid variables were included in the analysis the results were attenuated and the difference no longer significant. However, the trend towards a higher risk of heart failure was similar to that seen in the risk of CHD.

In the EURODIAB study, the risk of CHD in current smokers was similar to the risk in Study IV, although it was not statistically significant (78). In Study IV, when simple smoking status was used to group the participants, the risk in current smokers was similar to that in never smokers after adjustments for the lipid variables. The results in Study IV regarding the risk of heart failure in current smokers were in line with results based on data from the Swedish National Diabetes Register, although it combined current and former smokers (276).

To our knowledge, no previous studies have reported results regarding the effect of both intensity of smoking and cumulative smoking on the risk of different CVD outcomes in people with type 1 diabetes. In studies of the general population, the increased risk of CHD associated with current smoking is around 3 times higher compared with the risk seen in Study IV (357). However, this does not indicate that smoking is less harmful for people with diabetes. It is of note that all people with diabetes, regardless of smoking status, already have a 3–5-fold increased risk of CHD that will likely dilute the effect of smoking in a study including only people with diabetes.

Based on Study IV, smoking seems to have the strongest effect on the risk of stroke in people with diabetes, particularly in men. The risk of any stroke was doubled in men who were current smokers compared with never smokers. In the analysis including smoking intensity in packs per day and cumulative smoking in pack-years, the effect of smoking was more harmful on the risk of stroke compared with the risk of CHD or heart failure. Current smoking was not associated with a higher risk of stroke in women. However, in the general population smoking is shown to be equally harmful for men and women (270). The lack of a stronger association between smoking and the risk of stroke in women with diabetes could be explained by the fact that the excess risk of stroke associated with diabetes is around 30% higher in women than in men (177). Women also smoke less, and therefore the risk in all current smokers combined would presumably be lower. It is of note that there was no interaction between sex and smoking intensity or cumulative smoking in current smokers. This would indicate that the effect of smoking intensity and cumulative smoking is similar in men and women and that higher intensity or a cumulative dose is also harmful for women.

Previous studies have often combined the risk of different CVD outcomes, and only a few have reported results regarding the association between smoking and the risk of stroke in people with type 1 diabetes. In the WHO multinational study of vascular disease smoking was not a significant risk factor for stroke (274). This might be due to the lack of power in their study, as the number of stroke events among people with type 1 diabetes who were current smokers was low, 24 in men and 9 in women,

compared with 63 and 27 in Study IV. However, the trend in their results for the risk of stroke was similar to the results of Study IV regarding men who were current smokers.

7.6   Smoking cessation and the risk of diabetic nephropathy, coronary heart disease, heart failure, and stroke

The risk of diabetic nephropathy in former smokers was assessed using several different approaches. When all former smokers were combined in one group, the risk of any diabetic nephropathy or ESRD was not significantly higher compared with never smokers. However, in the analyses including pack-year data, the risk of microalbuminuria increased in former smokers by 3.4% per each smoked pack-year. The risk of macroalbuminuria and ESRD was also similar compared with never smokers in the analyses with cumulative smoking data. The higher microalbuminuria risk could be explained by the definition of microalbuminuria, which included three UAER values above the threshold. Some participants might have already stopped smoking before the first urine sample was above the normal limit, and if this happened before the baseline visit the participant was classified as a former smoker. Participants with one or two higher UAER values are clearly at higher risk of microalbuminuria compared with those with only normal UAER values. However, smoking cessation was beneficial regarding the further progression of microalbuminuria to macroalbuminuria or ESRD. This was also seen when former smokers were grouped according to the number of years they had not been smoking before the baseline visit. If former smokers had stopped smoking less than 5 years before the baseline visit, their risk was equal to the risk seen in current smokers. After 5–10 years, the risk gradually decreased to the same level as in never smokers.

Based on Study IV, the risk of CHD was similar in former smokers compared with never smokers when the association with simple smoking status was analyzed. However, we could observe a trend towards a higher risk in former smokers with a history of heavier smoking intensity, one pack of cigarettes more more per day. This was not the case in former smokers who had a history of heavier cumulative smoking or for the groups of former smokers who had smoked <20 or ≥20 pack-years, as they both had a similar risk of CHD as never smokers. In addition, if former smokers had an otherwise similar risk factor profile regarding glucose control, duration of diabetes, blood pressure, and lipids as never smokers, the risk of CHD did not differ from that for never smokers. These results are in line with those of the large EURODIAB study that reported a similar CHD risk in former and never smokers (78). Klein et al. reported a higher risk of myocardial

infarction in former smokers compared with never smokers, but they did not include the important risk factors of duration of diabetes, BMI, and lipids in their analysis (210).

The heart failure risk in Study IV was around 30% higher in former smokers compared with never smokers. This was especially seen in the analysis addressing the association between cumulative smoking and the risk of heart failure, and the risk increase was still significant after adjustments for the lipid variables. To our knowledge, this was the first study to report results regarding the association between former smoking and heart failure in people with type 1 diabetes. The very few previous studies did not report their results separately for former smokers, and in the study from the Swedish National Diabetes Register, current and former smokers were combined (276, 277).

In men, the risk of stroke remains twice as high compared with never smokers, even after smoking cessation. This risk was also higher after adjustments for other known CVD risk factors. This indicates that even though the CHD risk seems to decrease after smoking cessation, former smokers with type 1 diabetes are still at extremely high risk of CVD outcomes. In women, the risk of stroke did not differ between the former and never smokers. However, this should not be interpreted in such a way that the risk would be low in women who have stopped smoking. In fact, the risk of stroke is high in all women with type 1 diabetes, and former or current smoking does not change the risk significantly, partly because the smoking exposure in women is lower than in men (177). In the WHO multinational study of vascular disease, the risk of stroke was not increased in former smokers, men or women (274). This is in line with the Study IV results for women. As discussed previously, that study only had a small number of stroke events in former smokers, 8 in men and 5 in women. In Study IV, every single stroke event, including those that occurred after the CHD or heart failure outcomes, was registered. Therefore, the number of stroke events in Study IV is clearly higher, which might explain the differences in the results.

7.7   Study design, strengths, and weaknesses

One of the major strengths in Study I–Study IV is the large number of study participants and the precise information regarding alcohol consumption and smoking habits. Compared with the EURODIAB study regarding alcohol consumption and the risk of microvascular complications, the number of diabetic nephropathy and severe diabetic retinopathy cases was 4 times higher in Study I (332). The risk of diabetic nephropathy and severe diabetic retinopathy in former consumers of alcohol was evaluated separately, and thereby the "sick quitter" effect among abstainers was avoided. The

EURODIAB study also reported results regarding diabetic neuropathy, information that is not included in Study I. However, the specific effect of alcohol on diabetic neuropathy is difficult to assess given the fact that alcohol consumption is also an important cause of neuropathy in people without diabetes. Interpretation of the results of Study I is limited because of the cross-sectional nature of the study. The exact timeline for alcohol drinking in former consumers was also not available, and therefore it remains unknown if they changed their drinking pattern before or after the microvascular complication was diagnosed. However, the results regarding abstainers are more reliable in Study I compared with studies that have combined former drinkers and life-long abstainers. Binge drinking is associated with different vascular complications, and therefore information regarding drinking pattern might have elucidated the differences in the risk of diabetic nephropathy and severe diabetic retinopathy between consumers of different types of beverage.

Compared with previous studies on the association between smoking and vascular complications in people with type 1 diabetes, Study II and Study IV included a larger number of study participants and a longer follow-up time. In addition, most of the previous CVD studies only reported the results for CVD mortality or for the combined risk of any CVD. However, Study IV provides exact information regarding the effect of smoking on CHD, heart failure and stroke. Most of the previous studies reported results for never smokers compared with ever smokers, which in fact represents a combination of former and current smokers. Therefore, Study II and Study IV give valuable information about the risk of diabetic nephropathy and different CVD outcomes in former smokers.

The information about the participants' alcohol consumption and smoking habits was based on self-reported questionnaires, and no confirmative laboratory measurements, such as carbohydrate-deficient transferrin or phosphatidylethanol for alcohol consumption or serum or urine cotinine levels for smoking, were performed. However, based on previous observations, self-reported questionnaires give reliable estimates of alcohol intake for participants in epidemiological studies (358). In addition, smoking prevalence is more likely to be underestimated based on the questionnaire data, and therefore the effect of smoking would be underestimated rather than overestimated (359). The smoking data for Study IV were also revisited based on data from prospective visits and a follow-up questionnaire that was mailed to all FinnDiane participants in 2015. It is of note that the number of participants with incorrect baseline smoking data was low, and this information had to be corrected for only 46 participants. Exposure to passive smoking was not addressed in the questionnaire. This could have affected the results but mainly by increasing the risk of diabetic nephropathy or CVD risk in never

smokers, leading to a diminished difference between never smokers and current or former smokers.

Despite the additional information from the prospective visits and from the follow-up questionnaire, there was no access to exact data regarding smoking habits after the baseline visit and before each vascular event. Therefore, the effect of time-varying exposure to smoking during the follow-up was not taken into account. However, Study II and Study IV included excellent data regarding smoking habits before the baseline visit, and compared with previous studies, Study II and Study IV provide more precise data regarding the effect of intensity of smoking and cumulative smoking on the risk of vascular complications in people with type 1 diabetes.

To ensure correct alcohol and smoking data, participants with unclear information were excluded from the analyses. This might have affected the findings of Study I, as 579 (13.8%) participants were excluded due a to lack of alcohol consumption data. However, clearly, fewer participants were excluded due to lack of smoking data, namely 293 (7.5%) participants in Study II and 261 (5.5%) participants in Study IV. Only a few participants were excluded from the analysis because of unclear follow-up data regarding ESRD (4 participants) or CVD (4 participants) diagnoses. In Study II, Study III and Study IV, the diagnoses for ESRD and CVD outcomes were mainly based on register data from the Finnish Care Register for Health Care and the Cause of Death Register. In this study, only some ESRD diagnoses were collected from the medical records. However, register data regarding CHD has been verified previously in another FinnDiane study, and no classification errors were found (105).

7.8   Mechanisms behind the effect of smoking on vascular complications in people with diabetes

Cigarette smoke includes over 4000 possible harmful substances, and smoking affects the vasculature through multiple different pathways. Therefore, it is difficult to measure the independent effect of smoking on different vascular complications. In FinnDiane study population, current smokers had poorer glycemic control and a more atherosclerotic lipid profile with higher total cholesterol, triglycerides, and LDL cholesterol and lower HDL cholesterol. Generally, in CVD studies different risk factors are usually considered confounding factors. This is reasonable when, for example the association between a vascular complication and blood pressure is studied and the results are standardized for total cholesterol because high blood pressure does not directly elevate cholesterol values. However, regarding the associations between

smoking and different vascular complications, the results are more difficult to interpret. Based on the results of Study II and Study IV regarding the risk of diabetic nephropathy, CHD, heart failure, and stroke, the risk of any complication in question was increased in current smokers. However, after the results were adjusted for different lipid variables the increased risk was attenuated, and in some analyses the difference was no longer significant. Should this indicate that smoking is not a risk factor for those complications? A more likely scenario is that a part of the risk associated with smoking is actually mediated through the lipids.

In studies regarding risk factors for vascular complications in people with type 1 diabetes, the strongest risk factors are often other micro- or macrovascular complications. In Study IV regarding the risk of CHD, heart failure, and stroke, the effect of smoking was no longer significant after the results were adjusted for diabetic nephropathy. However, Study II showed that smoking increases the risk of any progression of diabetic nephropathy. Therefore, the fact that the effect of smoking on the risk of CVD is diluted when the results are adjusted for diabetic nephropathy does not indicate that smoking is a weaker risk factor for CVD. Instead, diabetic nephropathy could also be considered one of the mediating factors of the harmful effects of smoking.

Many other studies of people with type 1 diabetes have not been able to show significant associations between smoking and different vascular complications. Compared with Study II and Study IV, some of these studies were smaller and had shorter follow-up time. Many studies have also defined smokers differently by combining current and former smokers. They have also lacked the more accurate measures of intensity or cumulative smoking. In these studies, as well as in Study II and Study IV, the effect of smoking is decreased in the analyses combining many other risk factors. Indeed, if these risk factors are only interpreted as confounding factors the risk of smoking is hard to distinguish, especially in a smaller study population. However, if some of these risk factors, such as lipids, glucose control, and diabetic nephropathy, are interpreted as mediating factors, the overall risk associated with smoking is higher than the risk estimates would indicate, and smoking would actually be an even more important risk factor for vascular complications in people with type 1 diabetes.

8 SUMMARY AND CONCLUSIONS

I   People who have stopped their alcohol consumption have the highest risk of diabetic nephropathy and severe diabetic retinopathy. In addition, abstainers have a higher risk compared with people who are consuming alcohol. Compared with drinking wine, drinking spirits is associated with a higher risk of diabetic nephropathy in men and a higher risk of severe diabetic retinopathy in both sexes.

II Current smoking increases the risk of diabetic nephropathy, and the risk increases with the cumulative dose of smoking in pack-years. In former smokers, the risk of diabetic nephropathy is similar to that for never smokers as long as other risk factors are similar.

III In women, the SNP rs4972593 is a risk factor for ESRD comparable with smoking, but in non-smoking men the rs4972593 has a protective effect regarding the risk of ESRD.

IV Current smoking is associated with a higher risk of CHD and heart failure, particularly with higher cumulative smoking, and higher intensity of smoking. The risk of heart failure is increased in former smokers. In men, the risk of stroke is doubled in both current and former smokers compared with never smokers.

9 ACKNOWLEDGEMENTS

I want to express my deepest gratitude to my supervisor Professor Per-Henrik Groop, the founder of FinnDiane Study Group. His enthusiasm in the field of diabetes research has encouraged me and so many other young researchers during the past 20 years. It has been a privilege to work under his guidance. I am extremely grateful to my other supervisor Docent Valma Harjutsalo. I admire her knowledge in epidemiology and appreciate highly all the personal support and guidance she has given me during these years.

I owe my sincere gratitude to the official reviewers of this thesis, Professor Markus Juonala and Docent Satu Mäkelä. Their constructive comments and suggestions for improvements truly lifted this thesis to the next level.

I want to thank Carol Forsblom, the clinical co-ordinator of the FinnDiane Study, who has encouraged me to critical thinking in science. I value his excellent ideas and supportive guidance during these past years.

I also want to thank my other co-authors Lena Thorn, Raija Lithovius, Nina Elonen and Johan Wadén for their valuable contributions. Especially I would like to thank Niina Sandholm for guiding me through the complex field of genetics.

Espoo, December 2020

Maija Feodoroff

APPENDIX

The FinnDiane Study Centers	Physicians and nurses
Anjalankoski Health Center	S.Koivula, T.Uggeldahl
Central Finland Central Hospital, Jyväskylä	T.Forslund, A.Halonen, A.Koistinen, P.Koskiaho, M.Laukkanen, J.Saltevo, M.Tiihonen
Central Hospital of Åland Islands, Mariehamn	M.Forsen, H.Granlund, A.-C.Jonsson, B.Nyroos
Central Hospital of Kanta-Häme, Hämeenlinna	P.Kinnunen, A.Orvola, T.Salonen, A.Vähänen
Central Hospital of Kymenlaakso, Kotka	R.Paldanius, M.Riihelä, L.Ryysy
Central Hospital of Länsi-Pohja, Kemi	H.Laukkanen, P.Nyländen, A.Sademies
Central Ostrobothnian Hospital District, Kokkola	S.Anderson, B.Asplund, U.Byskata, P.Liedes, M.Kuusela, T.Virkkala
City of Espoo Health Center:	
Espoonlahti	A.Nikkola, E.Ritola
Tapiola	M.Niska, H.Saarinen
Samaria	E.Oukko-Ruponen, T.Virtanen
Viherlaakso	A.Lyytinen
City of Helsinki Health Center:	
Puistola	H.Kari, T.Simonen
Suutarila	A.Kaprio, J.Kärkkäinen, B.Rantaeskola
Töölö	P.Kääriäinen, J.Haaga, A-L.Pietiläinen
City of Hyvinkää Health Center	S.Klemetti, T.Nyandoto, E.Rontu, S.Satuli-Autere
City of Vantaa Health Center:	
Korso	R.Toivonen, H.Virtanen
Länsimäki	R.Ahonen, M.Ivaska-Suomela, A.Jauhiainen
Martinlaakso	M.Laine, T.Pellonpää, R.Puranen
Myyrmäki	A.Airas, J.Laakso, K.Rautavaara
Rekola	M.Erola, E.Jatkola
Tikkurila	R.Lönnblad, A.Malm, J.Mäkelä, E.Rautamo
Heinola Health Center	P.Hentunen, J.Lagerstam
Helsinki University Central Hospital, Department of Medicine, Division of Nephrology	A.Ahola, J.Fagerudd, M.Feodoroff, D.Gordin, O.Heikkilä, K.Hietala, M. Korolainen, J. Kytö, S. Lindh, K. Pettersson-Fernholm, M. Rosengård-Bärlund, M. Rönnback, A. Sandelin, A-R. Salonen, L. Salovaara, M. Saraheimo, JR Simonsen, L. Thorn, J. Tuomikangas, T. Vesisenaho, J. Wadèn
Herttoniemi Hospital, Helsinki	V.Sipilä
Hospital of Lounais-Häme, Forssa	T.Kalliomäki, J.Koskelainen, R.Nikkanen, N.Savolainen, H.Sulonen, E.Valtonen
Iisalmi Hospital	E.Toivanen
Jokilaakso Hospital, Jämsä	A.Parta, I.Pirttiniemi
Jorvi Hospital, Helsinki University Central Hospital	S.Aranko, S.Ervasti, R.Kauppinen-Mäkelin, A.Kuusisto, T.Leppälä, K.Nikkilä, L.Pekkonen
Jyväskylä Health Center, Kyllö	K.Nuorva, M.Tiihonen

Kainuu Central Hospital, Kajaani	S.Jokelainen, P.Kemppainen, A-M.Mankinen, M.Sankari
Kerava Health Center	H.Stuckey, P.Suominen
Kirkkonummi Health Center	A.Lappalainen, M.Liimatainen, J.Santaholma
Kivelä Hospital, Helsinki	A.Aimolahti, E.Huovinen
Koskela Hospital, Helsinki	V.Ilkka, M.Lehtimäki
Kotka Health Center	E.Pälikkö-Kontinen, A.Vanhanen
Kouvola Health Center	E.Koskinen, T.Siitonen
Kuopio University Hospital	E.Huttunen, R.Ikäheimo, P.Karhapää, P.Kekäläinen, M.Laakso, T.Lakka, E.Lampainen, L.Moilanen, L.Niskanen, U.Tuovinen, I.Vauhkonen, E.Voutilainen
Kuusamo Health Center	T.Kääriäinen, E.Isopoussu
Kuusankoski Hospital	E.Kilkki, I.Koskinen, L.Riihelä
Laakso Hospital, Helsinki	T.Meriläinen, P.Poukka, R.Savolainen, N.Uhlenius
Lahti City Hospital	A.Mäkelä, M.Tanner
Lapland Central Hospital, Rovaniemi	L.Hyvärinen, S.Severinkangas, T.Tulokas
Lappeenranta Health Center	P.Linkola, I.Pulli
Lohja Hospital	T.Granlund, M.Saari, T.Salonen
Länsi-Uusimaa Hospital, Tammisaari	I.-M.Jousmaa, J.Rinne
Loimaa Health Center	A.Mäkelä, P.Eloranta
Malmi Hospital, Helsinki	H.Lanki, S.Moilanen, M.Tilly-Kiesi
Mikkeli Central Hospital	A.Gynther, R.Manninen, P.Nironen, M.Salminen, T.Vänttinen
Mänttä Regional Hospital	I.Pirttiniemi, A-M.Hänninen
North Karelian Hospital, Joensuu	U-M.Henttula, P.Kekäläinen, M.Pietarinen, A.Rissanen, M.Voutilainen
Nurmijärvi Health Center	A.Burgos, K.Urtamo
Oulaskangas Hospital, Oulainen	E.Jokelainen, P.-L.Jylkkä, E.Kaarlela, J.Vuolaspuro
Oulu Health Center	L.Hiltunen, R.Häkkinen, S.Keinänen-Kiukaanniemi
Oulu University Hospital	R.Ikäheimo
Päijät-Häme Central Hospital	H.Haapamäki, A.Helanterä, S.Hämäläinen, V.Ilvesmäki, H.Miettinen
Palokka Health Center	P.Sopanen, L.Welling
Pieksämäki Hospital	V.Javtsenko, M.Tamminen
Pietarsaari Hospital	M-L.Holmbäck, B.Isomaa, L.Sarelin
Pori City Hospital	P.Ahonen, P.Merensalo, K.Sävelä
Porvoo Hospital	M.Kallio, B.Rask, S.Rämö
Raahe Hospital	A.Holma, M.Honkala, A.Tuomivaara, R.Vainionpää
Rauma Hospital	K.Laine, K.Saarinen, T.Salminen
Riihimäki Hospital	P.Aalto, E.Immonen, L.Juurinen
Salo Hospital	A.Alanko, J.Lapinleimu, P.Rautio, M.Virtanen
Satakunta Central Hospital, Pori	M.Asola, M.Juhola, P.Kunelius, M.-L.Lahdenmäki, P.Pääkkönen, M.Rautavirta
Savonlinna Central Hospital	T.Pulli, P.Sallinen, M.Taskinen, E.Tolvanen, H.Valtonen, A.Vartia
Seinäjoki Central Hospital	E.Korpi-Hyövälti, T.Latvala, E.Leijala
South Karelia Central Hospital, Lappeenranta	T.Ensala, E.Hussi, R.Härkönen, U.Nyholm, J.Toivanen
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Tiirismaa Health Center, Hollola	T.Kivelä, L.Petlin, L.Savolainen
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Turku University Central Hospital	K.Breitholz, R.Eskola, K.Metsärinne, U.Pietilä, P.Saarinen, R.Tuominen, S.Äyräpää
Vaajakoski Health Center	K.Mäkinen, P.Sopanen
Valkeakoski Regional Hospital	S.Ojanen, E.Valtonen, H.Ylönen, M.Rautiainen, T.Immonen
Vammala Regional Hospital	I.Isomäki, R.Kroneld, M.Tapiolinna-Mäkelä
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