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INCIDENCE, PROGRESSION, AND REGRESSION OF DIABETIC KIDNEY DISEASE IN TYPE 1 DIABETES

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Doctoral dissertation

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“It is simply this: do not tire, never lose interest, never grow indifferent –
lose your invaluable curiosity and you let yourself die.
It's as simple as that.”

Tove Jansson

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ABSTRACT

Background Despite improved treatment strategies during the past decades, individuals with type 1 diabetes remain burdened with an increased risk of premature mortality, especially due to cardiovascular causes of death. The presence and severity of diabetic kidney disease (DKD) is a main driver of the risk. DKD is predicted to affect every third individual with type 1 diabetes; however, the contemporary natural history of DKD has been poorly described.

Aim The aim of this doctoral thesis was to obtain a holistic view of incidence, progression, and regression of DKD. Its progression was assessed in light of remnant cholesterol and apolipoprotein C-III (apoC-III), both key components of the triglyceride-rich lipoprotein metabolism.

Methods We studied incidence rate patterns, cumulative incidences, and time trends of moderate and severe albuminuria (the first clinical stages of DKD) using a population-based study design. The cohort encompassed a stratified random sample (n=1,500) of all individuals diagnosed with type 1 diabetes at the age of 0-14 years during 1970-99 in Finland. Differences between three cohorts defined by the calendar year of diabetes diagnosis (1970-79, 1980-89, 1990-99) were assessed. We systematically reviewed medical records retrieved from the onset of diabetes up to 31 December 2020 for albuminuria determinations. Moderate albuminuria was explored in those with diabetes onset in 1980-99, and severe albuminuria in the whole population. Progression and regression of albuminuria were assessed in the nationwide, multicenter Finnish Diabetic Nephropathy (FinnDiane) Study. Progression was defined as a change to a more advanced stage of DKD and was ascertained from medical records and national registers. Remnant cholesterol was calculated as total cholesterol minus HDL-cholesterol minus LDL-cholesterol, whereas the apoC-III concentration was measured directly. We evaluated the association between remnant cholesterol, apoC-III and DKD progression. We also assessed the impact of DKD on the association between remnant cholesterol, apoC-III, cardiovascular events, and mortality. Regression of albuminuria was defined as a change to a less advanced stage of albuminuria before the FinnDiane baseline visit. We investigated the association between regression and incident cardiovascular events and mortality.

Results The diabetes duration-specific incidence rate pattern of severe albuminuria had changed over time; the incidence peak noted at 15-19 years of diabetes duration in the 1970-79 diagnosis cohort was not replicated in those diagnosed later. In the combined 1980-99 calendar-year cohort, the incidence rate increased during the first 14 years of diabetes, after which it levelled off to a plateau. The cumulative incidence of severe albuminuria had

approximately halved between the 1970-79 and 1980-89 cohorts, but no further decrease was noticed between 1980-89 and 1990-99. The cumulative progression rate between severe albuminuria and kidney failure had also not changed over time. The incidence rate of moderate albuminuria increased until 10 years after diabetes onset, then remained stable until starting to decrease at around 25 years of duration. No signs of a calendar effect for moderate albuminuria between 1980-89 and 1990-99 appeared. The albuminuria regression rate was 23.3% and 23.4% from moderate and severe albuminuria, respectively, in the FinnDiane cohort. Regression of albuminuria was associated with reduced risk of cardiovascular events and mortality to the same level of those who did not progress in the first place. Remnant cholesterol was robustly associated with DKD progression and was also a powerful predictor of future cardiovascular events and mortality, except in the study participants with kidney failure. Regarding DKD, apoC-III was primarily associated with the progression from moderate to severe albuminuria, and the association between apoC-III and cardiovascular disease/mortality was limited to those with albuminuria. However, neither of these associations was independent of remnant cholesterol.

Conclusions The incidence of albuminuria has decreased over time but has plateaued after the 1980s. Hence, a substantial residual burden of DKD in type 1 diabetes remains and means to tackle it are needed. Regression of albuminuria is a frequent phenomenon, and it is associated with an overall improved prognosis. The triglyceride-rich lipoprotein metabolism appears to be implicated in the development of various vascular diseases in type 1 diabetes, with remnant cholesterol possibly mediating the effects of apoC-III.

ABSTRAKT (ABSTRACT IN SWEDISH)

Bakgrund Trots att behandlingsmöjligheterna förbättrats under de senaste decennierna belastas individer med typ 1-diabetes fortfarande med en ökad risk för förtidig död, i synnerhet på grund av kardiovaskulära dödsorsaker. Denna risk styrs till en stor grad av förekomsten och svårighetsgraden av diabetisk njursjukdom. Diabetisk njursjukdom uppskattas drabba var tredje person med typ 1-diabetes, men sjukdomens nutida förlopp är inte fullständigt skildrat.

Målsättning Syftet med denna doktorsavhandling var att erhålla en helhetsbild av incidensen, progressionen och regressionen av diabetisk njursjukdom. Progressionen bedömdes utifrån restkolesterol och apolipoprotein C-III (apoC-III), som är nyckelkomponenter i de triglyceridrika lipoproteinernas metabolism.

Metoder Vi analyserade incidensmönster, kumulativa incidenser och tidstrender av ökad och tydligt ökad albuminuri (de första kliniska stadierna av diabetisk njursjukdom) utifrån en populationsbaserad studiedesign. Kohorten omfattade ett stratifierat slumpmässigt urval (n=1500) av alla individer som diagnostiserats med typ 1-diabetes i åldern 0-14 år under 1970-99 i Finland. Skillnader i kohorter definierade efter kalenderår för diabetesdiagnos (1970-79, 1980-89 och 1990-99) studerades. Albuminurimätningarna erhölls genom systematisk granskning av sjukjournaler fram till den 31.12.2020. Ökad albuminuri undersöktes hos de personer som insjuknat i diabetes under 1980-99 och tydligt ökad albuminuri i hela kohorten. Progression och regression av albuminuri utvärderades i den nationella multicenterstudien Finnish Diabetic Nephropathy (FinnDiane) Study. Progression definierades som en förändring till ett mer framskridet stadium av njursjukdom och fastställdes från sjukjournaler och nationella register. Restkolesterolet kalkylerades enligt totalkolesterol minus HDL-kolesterol minus LDL-kolesterol, emedan apoC-III-koncentrationen mättes direkt. Vi analyserade sambandet mellan restkolesterol, apoC-III och progression av diabetisk njursjukdom. Vi studerade även effekten av diabetisk njursjukdom på sambandet mellan restkolesterol, apoC-III, kardiovaskulära händelser och mortalitet. Regression av albuminuri definierades som en förändring till ett mindre framskridet stadium av albuminuri innan det första FinnDiane-besöket. Vi utvärderade sambandet mellan regression och kardiovaskulära händelser samt mortalitet.

Resultat Det diabetesdurationsspecifika incidensmönstret för tydligt ökad albuminuri hade förändrats med tiden; incidenstoppen vid 15-19 år av diabetesduration som framkom i 1970–79-kohorten kunde inte längre

observeras hos dem som diagnostiserats senare. I den kombinerade diagnosårskohorten 1980–99 steg incidensen under de första 14 åren av diabetes, varefter den planade ut till en platå. Mellan 1970-79 och 1980-89 hade den kumulativa incidensen av tydligt ökad albuminuri ungefär halverats, emedan mellan 1980-89 och 1990-99 kunde ingen ytterligare förminskning noteras. Den kumulativa progressionshastigheten mellan tydligt ökad albuminuri och njursvikt hade heller inte förändrats. Incidensen av ökad albuminuri steg under de första 10 åren av diabetes och förblev sedan stabil, ända tills den började avta kring 25 år av diabetesduration. Den kumulativa incidensen av ökad albuminuri påvisade inga tecken av en kalendereffekt mellan 1980-89 och 1990-99. I FinnDiane-kohorten var graden av albuminuriregression 23,3 % från ökad albuminuri och 23,4 % från tydligt ökad albuminuri. Regressionen var associerad med en riskminskning av kardiovaskulära händelser och mortalitet till samma risknivå som drabbade dem vars njursjukdomsstadium ursprungligen inte hade framskridit. Restkolesterolskoncentrationen var starkt associerad med progression av diabetisk njursjukdom och, förutom hos studiedeltagarna med njursvikt, även med framtida kardiovaskulära händelser och mortalitet. Angående diabetisk njursjukdom var apoC-III främst associerad med progressionen från ökad till tydligt ökad albuminuri, och sambandet mellan apoC-III och kardiovaskulär sjukdom/mortalitet begränsades till de personer som hade albuminuri. Inget av dessa samband var dock oberoende av restkolesterolskoncentrationen.

Slutsatser Incidensen av albuminuri har minskat med tiden, men efter 1980-talet har förminskningen uppnått en platåfas. Därmed kvarstår diabetisk njursjukdom som en betydelsefull börda förknippad med typ 1-diabetes, och ytterligare åtgärder för att tackla bördan krävs. Regression av albuminuri är ett frekvent fenomen som associeras med en förbättrad prognos hos patienterna. De triglyceridrika lipoproteinernas metabolism verkar vara inblandad i utvecklingen av olika vaskulära sjukdomar vid typ 1-diabetes, och det är möjligt att restkolesterolet förmedlar effekterna av apoC-III.

TIIVISTELMÄ (ABSTRACT IN FINNISH)

Tausta Viimeisten vuosikymmenien aikana tyypin 1 diabeteksen hoitostrategiat ovat parantuneet. Tästä huolimatta tautia sairastavia henkilöitä kuormittaa edelleen lisääntynyt ennenaikaisen kuolleisuuden riski, etenkin riski kuolla kardiovaskulaaritauteihin. Kohonneesta riskistä kertovat erityisesti diabeteksen munuaistaudin esiintyminen ja sen vaikeusaste. Diabeteksen munuaistaudin ennustetaan kehittyvän joka kolmannelle tyypin 1 diabetesta sairastavalle henkilölle, mutta nykyinen taudin tyypillinen kulku ei ole täysin selvä.

Tavoite Tämän väitöskirjan tavoitteena oli selvittää kokonaiskuva diabeteksen munuaistaudin ilmaantuvuudesta, progressiosta ja regressiosta. Diabeteksen munuaistaudin progressiota tutkittiin tarkastellen jäännöskolesterolia ja apolipoproteiini C-III:a (apoC-III), jotka ovat triglyseridipitoisen lipoproteiiniaineenvaihdunnan keskeisiä osatekijöitä.

Menetelmät Tutkimme lisääntyneen ja selvästi lisääntyneen albuminurian (diabeteksen munuaistaudin ensimmäisten kliinisten vaiheiden) ilmaantuvuuskuvioita, kumulatiivisia ilmaantuvuuksia sekä ajallisia trendejä väestöpohjaisessa tutkimuksessa. Tutkimuskohortti koostui ositetusta satunnaisotoksesta (n=1500) kaikista tyypin 1 diabetekseen 0-14 vuoden iässä sairastuneista henkilöistä vuosina 1970-1999 Suomessa. Tutkimme eroja kolmen kohortin välillä, jotka oli määritelty diabetesdiagnoosin kalenterivuoden mukaan (1970-79, 1980-89 ja 1990-99) välillä. Albuminuriamääritykset tarkastettiin sairauskertomusmerkinnöistä 31.12.2020 asti. Lisääntyneen albuminurian ilmaantuvuutta tutkittiin vuosina 1980-99 diabetekseen sairastuneilta henkilöiltä ja selvästi lisääntynyttä albuminuriaa koko väestöstä. Albuminurian progressiota ja regressiota arvioitiin valtakunnallisessa Finnish Diabetic Nephropahy (FinnDiane) Study -monikeskustutkimuksessa. Progressio määriteltiin munuaistaudin luokituksen etenemisenä korkeammalle tasolle ja varmistettiin sairauskertomuksista ja kansallisista rekistereistä. Jäännöskolesteroli laskettiin kaavalla kokonaiskolesteroli miinus HDL-kolesteroli miinus LDL-kolesteroli, kun taas apoC-III:n pitoisuus selvitettiin suoralla mittauksella. Tutkimme jäännöskolesterolin, apoC-III:n ja munuaistaudin progression välistä yhteyttä. Selvitimme myös munuaistaudin vaikutusta jäännöskolesterolin, apoC-III:n, kardiovaskulaaritapahtumien sekä kuolleisuuden väliseen yhteyteen. Albuminurian regressio määriteltiin munuaistaudin luokituksen palaamisena vähemmän edenneelle tasolle ennen FinnDianen ensimmäistä tutkimuskäyntiä. Tutkimme regression ja kardiovaskulaaritapahtumien sekä kuolleisuuden välistä yhteyttä.

Tulokset Selvästi lisääntyneen albuminurian ilmaantumistiheys diabeteksen keston suhteen oli muuttunut ajan saatossa; 1970-1979-kohortissa todettiin ilmaantuvuushuippu 15-19 vuoden kohdalla diabeteksen puhkeamisesta, mutta tämä huippu ei enää ollut havaittavissa myöhemmin diagnosoiduilla. Yhdistetyssä diagnoosivuosien 1980-1999 kohorteissa ilmaantuvuus nousi ensimmäisten 14 vuoden aikana diabeteksen puhkeamisen jälkeen, jonka jälkeen se tasaantui. Selvästi lisääntyneen albuminurian kumulatiivinen ilmaantuvuus puolittui diagnoosikohorttien 1970-79 ja 1980-89 välillä, kun taas 1980-89 ja 1990-99 välillä ei ollut havaittavissa muutosta. Lisäksi selvästi lisääntyneen albuminurian etenemisnopeus munuaisten vajaatoiminnaksi ei ollut muuttunut. Lisääntyneen albuminurian kumulatiivinen ilmaantuvuus ei osoittanut merkkejä kalenterivaikutuksesta vuosien 1980-89 ja 1990-99 välillä. Lisääntyneen albuminurian ilmaantuvuus nousi 10 vuoden ajan pysyen sen jälkeen vakaana, kunnes se alkoi laskea noin 25 vuoden kohdalla diabeteksen puhkeamisesta. Albuminurian regressioaste FinnDiane-kohortissa lisääntyneestä albuminuriasta oli 23,3 % ja selvästi lisääntyneestä albuminuriasta 23,4 %. Albuminurian regressioon liittyi kardiovaskulaaritapahtumien riskin sekä kuolleisuuden riskin lasku vähemmän edenneen munuaistaudin luokitusta vastaavalle tasolle. Jäännöskolesterolin pitoisuus liittyi vahvasti munuaistaudin etenemiseen. Se ennusti myös kardiovaskulaaritapahtumia ja kuolleisuutta, paitsi niillä tutkittavilla, joilla oli munuaisten vajaatoiminta. Munuaistaudin osalta apoC-III:n pitoisuus oli lähinnä yhteydessä lisääntyneen albuminurian progressioon ja sen yhteys kardiovaskulaaritauteihin/kuolleisuuteen rajoittui vain niihin henkilöihin, joilla oli albuminuria. Mikään näistä yhteyksistä ei kuitenkaan ollut riippumaton jäännöskolesterolista.

Päätelmät Albuminurian ilmaantuvuus on vähentynyt ajan saatossa, mutta lasku on tasaantunut 1980-luvun jälkeen. Näin ollen munuaistauti on edelleen merkittävä riski tyypin 1 diabeteksessa, ja toimenpiteitä sen taltuttamiseksi tarvitaan. Albuminurian regressio on yleistä ja tähän liittyy parantunut kokonaisennuste. Triglyseridirikkaiden lipoproteiinien aineenvaihdunta näyttää olevan osallisena erinäisten verisuonisairauksien kehittymiseen tyypin 1 diabeteksessa. ApoC-III saattaa vaikuttaa näiden tautien etenemiseen jäännöskolesterolin kautta.

LIST OF ORIGINAL PUBLICATIONS

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I Jansson Sigfrids F, Groop P-H, Harjutsalo V. Incidence rate patterns, cumulative incidence, and time trends for moderate and severe albuminuria in individuals diagnosed with type 1 diabetes aged 0-14 years: a population-based retrospective cohort study *Lancet Diabetes & Endocrinology*. 2022. Epub ahead of print.

II Jansson Sigfrids F, Dahlström EH, Forsblom C, Sandholm N, Harjutsalo V, Taskinen M-R, Groop P-H. Remnant cholesterol predicts progression of diabetic nephropathy and retinopathy in type 1 diabetes. *Journal of Internal Medicine*. 2021;290:632-645

III Jansson Sigfrids F*, Stechemesser L*, Dahlström EH, Forsblom C, Harjutsalo V, Taskinen M-R, Groop P-H. Apolipoprotein C-III predicts cardiovascular events and mortality in individuals with type 1 diabetes and albuminuria. *Journal of Internal Medicine*. 2022;291:338-349

IV Jansson FJ, Forsblom C, Harjutsalo V, Thorn LM, Wadén J, Elonen N, Ahola AJ, Saraheimo M, Groop P-H. Regression of albuminuria and its association with incident cardiovascular outcomes and mortality in type 1 diabetes: the FinnDiane Study. *Diabetologia* 2018;61:1203-1211

* Equal contribution

Henceforward, the publications are referred to by their Roman numerals.

ABBREVIATIONS

95% CI	95% confidence interval
ACE	Angiotensin-converting enzyme
ADA	American Diabetes Association
ACR	Albumin-creatinine-ratio
AER	Albumin excretion rate
Apo	Apolipoprotein
ARB	Angiotensin II receptor blocker
BMI	Body-mass index
CAD	Coronary artery disease
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CV	Coefficient of variation
CVD	Cardiovascular disease
DCCT	Diabetes Control and Complications Trial
DKD	Diabetic kidney disease
EDC	Epidemiology of Diabetes Complications
EDIC	Epidemiology of Diabetes Interventions Study
eGFR	Estimated glomerular filtration rate
FinnDiane	Finnish Diabetic Nephropathy
GAM	Generalized additive model
GFR	Glomerular filtration rate
HbA_{1c}	Glycated hemoglobin A_{1c}
HDL	High-density lipoprotein
HLA	Human leukocyte antigen
HR	Hazard ratio
IDL	Intermediate-density lipoprotein
IQR	Interquartile range
KDIGO	Kidney Disease: Improving Global Outcomes
LDL	Low-density lipoprotein
LPL	Lipoprotein lipase
LRP	LDL-receptor-related protein
mGFR	Measured glomerular filtration rate
RAAS	Renin-angiotensin-aldosterone system
ROS	Reactive oxygen species
SGLT2	Sodium-glucose cotransporter 2
TRL	Triglyceride-rich lipoprotein
VLDL	Very-low density lipoprotein
WHR	Waist-to-hip ratio

1 INTRODUCTION

The rampant epidemic of diabetes is a global health emergency and one of the fastest-growing disease burdens of the 21st century. In 2021, 537 million adults aged 20-79 years were already afflicted with diabetes, yet the number is predicted to reach 783 million by 2045 [1]. Nearly half a million Finns are currently living with a diabetes diagnosis, of whom ~10% have its insulin-dependent, autoimmune form referred to as type 1 diabetes [2]. A rise in the incidence of type 1 diabetes since the 1950s has been reported [3,4], and the worldwide peak incidence of the disease was achieved in Finland in 2006 [5]. Whereas the increase of type 2 diabetes is thought to mirror the rising prevalence of obesity and sedentary behavior, the reasons for the uncontrolled upsurge of type 1 diabetes are incompletely understood. However, a complex interplay between environmental and genetic factors is presumed [6].

People with type 1 diabetes remain burdened with high premature mortality risk despite vast advances in treatment strategies over the past 40 years. Acute diabetic complications drive the mortality during the first decade of diabetes [7,8], whereas cardiovascular causes of death prevail thereafter [8–10]. Even in the 21st century, the life expectancy of young adults with type 1 diabetes has been projected to be 10 to 12 years shorter than that of their diabetes-free sex- and age-matched counterparts [11]. However, the mortality risk within the type 1 diabetes population is uneven. While many factors influence the prognosis, the presence and severity of diabetic kidney disease (DKD) is the key determinant of the risk [12,13].

DKD, a microvascular chronic diabetes complication, has traditionally been estimated to affect 25 to 40% of individuals with type 1 diabetes after 20 to 25 years [14–16]. However, most of the epidemiological reports stating this go back at least 40 years, that is, to an era when blood glucose self-monitoring had only recently been introduced and the impact of blood pressure control on the DKD risk was only hinted at. The more recent analyses have commonly been limited by critical shortcomings, such as single-center study designs, small-scale study cohorts, or short observation times. The incidence of moderate albuminuria, the first clinical stage of DKD, has been particularly poorly described. Furthermore, the pre-existing studies have been restricted to individuals with diabetes diagnosed in the 1980s or the preceding decades.

The contemporary natural course of DKD after the onset of albuminuria is neither fully recognized. Early reports deemed DKD to be a progressive disease, evolving via rising albuminuria to kidney failure within a short time span [17–19]. Nevertheless, such a view of inexorable DKD progression has been doubted over the past years as regression of albuminuria has also been observed. The reported regression rates have ranged between 15 and 70%, with substantial discrepancies in study designs and cohorts. A particular gap in the knowledge remains for the clinical consequences of regression: It is not known

whether and to what extent regression of albuminuria affects the risk of cardiovascular disease (CVD) and mortality in type 1 diabetes.

Many studies have aimed to unravel the risk factors of DKD to identify high-risk individuals at an early stage to, hopefully, be able to prevent the development or to halt the progression of the disease. Compelling evidence is pointing towards both genetic and environmental culprits. Along with suboptimal glycemic control and hypertension, dyslipidemia has emerged as a pivotal modifiable DKD risk factor, with hypertriglyceridemia showing the strongest association [20]. Hypertriglyceridemia has also been linked to an increased risk of CVD within this patient population [21]. However, with respect to atherosclerosis, the current understanding is that the triglycerides act as a surrogate marker of triglyceride-rich lipoproteins (TRLs) and their remnant cholesterol, which accumulates in the atherosclerotic plaques – whereas triglycerides, *per se*, do not [22]. A recent intriguing report revealed a robust relationship between CVD and apolipoprotein C-III (apoC-III), a key regulator of the TRL metabolism, in a small type 1 diabetes cohort [23]. However, whether TRL remnant cholesterol and apoC-III predict the development and progression of DKD in type 1 diabetes is undisclosed.

The aim of this doctoral thesis was to present a contemporary and comprehensive view of the natural history of DKD – its incidence, progression, and regression. Incidence rate patterns, cumulative incidences, and time trends for moderate and severe albuminuria were studied in a population-based cohort of individuals with type 1 diabetes. The course of albuminuria was assessed in the nationwide, multicenter Finnish Diabetic Nephropathy (FinnDiane) Study. The progression of DKD was evaluated in view of the TRL metabolism and some of its main components. Finally, the rate and clinical consequences of albuminuria regression were established.

2 REVIEW OF THE LITERATURE

2.1 Type 1 diabetes

2.1.1 Definition and classification of diabetes

Diabetes mellitus is a heterogeneous group of metabolic disorders with a shared feature, hyperglycemia, secondary to either inadequate insulin secretion, insulin resistance, or both. The criteria for diagnosing diabetes are a fasting plasma glucose ≥7.0 mmol/L (126 mg/dL); plasma glucose ≥11.1 mmol/L (≥200 mg/dL) 2 hours after a 75 g glucose load (oral glucose tolerance test); or glycated hemoglobin A_{1c} (HbA_{1c}) ≥48 mmol/mol (≥6.5%) using a standardized assay. The diagnosis requires two abnormal test results either from the same or from two separate test samples in asymptomatic individuals, whereas one test result above the diagnostic level is sufficient in individuals who present with symptoms of hyperglycemia. A random plasma glucose of 11.1 mmol/L or higher is also diagnostic for diabetes in symptomatic individuals [24]. Diabetes can be subdivided into several distinct diseases based on clinical characteristics, C-peptide as an indicator of insulin secretion, the presence or absence of autoantibodies, and genetics. These comprise gestational diabetes, latent autoimmune diabetes in adults (LADA), maturity-onset diabetes of the young (MODY), other rare monogenic forms such as neonatal diabetes, and secondary diabetes (*e.g.,* due to pancreatic diseases). A novel classification of adult-onset diabetes has also been proposed during recent years [25]. However, most of the over 500 million individuals with chronic hyperglycemia exceeding the diabetic threshold are to date categorized as having type 2 (~90%) or type 1 diabetes (5 to 10%) [24].

Type 1 diabetes is characterized by immune-mediated destruction of insulin-producing pancreatic β-cells and subsequent lifelong dependence on exogenous insulin. The following sections will focus on the epidemiology and natural history of type 1 diabetes and, subsequently, on the vicious, long-term complications of the disease.

2.1.2 Epidemiology

Diabetes has a long history: The earliest traceable remarks relating to manifestations of hyperglycemia date back ~3,500 years to ancient Egypt. The Greek physician and philosopher Galen portrayed the condition as a disease of the kidneys in the first century *AD*, and this understanding was supported by many experts during succeeding years. It was not until the latter part of the 19th century that diabetes mellitus was linked to the pancreas and, even later,

that DKD was identified as a separate entity [26]. Type 1 diabetes was a terminal disease until the discovery of insulin in 1921 and the first successful human administration of purified canine pancreatic extract in 1922 [27], leading to death usually within the span of months.

In the absence of rigorous epidemiological studies, there is scant evidence of the incidence and prevalence of type 1 diabetes in the pre-insulin era and the immediately following years. However, specialized clinic series and mortality statistics, reviewed by Professor Gale [3], suggest that type 1 diabetes was rather rare (incidence rate ~2-7/100,000/year) at the turn of the 20th century. These reports, however, almost certainly underestimate actual frequencies and must therefore be cautiously interpreted.

A steady growth in the incidence of type 1 diabetes became a global phenomenon around the 1950s [3]. A systematic review covering studies from nearly 30 countries, focusing on time trends in children aged 0-14 years during 1960-1996, revealed a mean annual increase of 3.0% with even higher relative growth in countries with low incidence rates at baseline [4]. Nevertheless, the difference between the populations with the highest (Sardinia, Finland) and lowest (China) incidence rates was more than 350-fold [28] in the 1990s.

Insulin treatment became free of charge in Finland in 1964, which has enabled the ascertainment of almost all new cases of type 1 diabetes through insulin reimbursement applications since then. The incidence of type 1 diabetes rose by 2.4% per annum between 1965 and 1984 [29] and by 3.0% in 1980 to 2005 [30] in the 0-14-year group, reaching a peak in 2006 when the global all-time highest incidence was achieved (64.9/100,000/year) [5]. This acceleration has, fortunately, been followed by a plateau [5], and a recent Finnish study even suggested that the incidence may be declining in the youngest age group (0-4 years) [31].

The epidemiological reports on type 1 diabetes – especially the newer ones and those portraying secular trends – are primarily confined to individuals with childhood-onset (0-14 years) disease. For instance, the most recent type 1 diabetes incidence analysis of young adults (15-39 years) in Finland dates back over 20 years [32]. The lack of updates in adult populations is striking, because although the incidence peaks during puberty [33,34], type 1 diabetes can develop throughout the lifespan, and onset in adulthood is actually more prevalent than onset in childhood [35]. In contrast, especially at higher onset ages, type 1 diabetes is a heterogeneous disease, and results drawn from an adult-onset or a mixed population might not apply to those with childhood-onset disease. Therefore, it is essential to distinguish by the onset age in epidemiological studies on type 1 diabetes and its complications.

2.1.3 Pathogenesis

The progression to type 1 diabetes is a complex interplay between genes and environmental triggers. Three distinct phases can be identified, although the asymptomatic prodrome is considered a continuum [24,36].

The first phase is characterized by normoglycemia and the persistent presence of two or more autoantibodies. Several autoantibodies against islet antigens have been associated with type 1 diabetes [37]. These act as markers, rather than mediators, of ongoing humoral beta-cell autoimmunity. Seroconversion occurs in most individuals before the age of four, with a peak during the second year of life [38].

Individuals in the second phase are still asymptomatic but have developed dysglycemia arising from the advancing T-cell-mediated destruction of β-cells. The risk of progression to overt diabetes is high at this stage, especially in young children [39]. Phase III is the first symptomatic stage, during which the clinical manifestations of diabetes, such as polyuria, polydipsia, fatigue, or weight loss, evolve. The duration from seroconversion to the symptomatic stage ranges from months to decades [36].

Familial clustering of type 1 diabetes occurs despite most individuals with type 1 diabetes not having a family history of the disease; the proband-wise concordance rate in monozygotic twins is ~40% [40], the sibling recurrence risk 5-6% [41], and the offspring recurrence risk 4-8% in the offspring of male probands and 2-5% in the offspring of female probands [42–44]. Nearly 80 genetic loci have been associated with type 1 diabetes [45], and the human leukocyte antigen (HLA) region that maps to chromosome 6p21 is a particularly important contributor to the genetic susceptibility of type 1 diabetes [46]. This region encodes the major histocompatibility complex I and II proteins that present antigens to T-lymphocytes. The main genetic determinants of type 1 diabetes risk are specific combinations of HLA class II DR-DQ associations with both susceptible, neutral, and protective haplotypes [46].

However, along with the steep rise in type 1 diabetes incidence [4], the prevalence of high-risk HLA genotypes has declined, whereas protective genotypes have become more common [47]. These findings together support a greater contribution of environmental factors in the contemporary natural history of type 1 diabetes. Insufficient early childhood microbial exposure [48] but also early-childhood infections (particularly enterovirus infections) [49], vitamin D deficiency [50], intestinal dysbiosis [51], and maternal overweight and obesity [52], among others, have emerged as putative exogenous triggers.

2.1.4 Complications

Type 1 diabetes can give rise to acute and chronic complications. Possible acute complications include hypoglycemia, diabetic ketoacidosis, and hyperosmolar

hyperglycemic state. The two latter are hyperglycemic emergencies that develop in a milieu of insulin deficiency and concomitant increase in the counter-regulatory hormones, leading to decreased glucose utilization, accelerated gluconeogenesis, glycogenolysis, and, consequently, hyperglycemia. In diabetic ketoacidosis, increased lipolysis results in an abundance of free fatty acids that are converted to acidic ketone bodies. Metabolic acidosis, exaggerated by fluid and electrolyte loss, evolves. The hyperosmolar hyperglycemic state is overall rarer than diabetic ketoacidosis and occurs more often in type 2 than in type 1 diabetes. It is characterized by hyperglycemia without significant ketosis and acidosis. All the acute complications of type 1 diabetes are possibly life-threatening conditions. [53]

The chronic or long-term complications of type 1 diabetes can be sub-divided into micro- and macrovascular diseases, depending on the size of the blood vessels they affect. The blood vessels of the kidney, the retina, or the nervous system are diseased in diabetic microvascular disease. Macrovascular complications involve larger blood vessels, resulting in coronary artery disease (CAD), cerebrovascular disease, or peripheral artery disease. The following chapter compiles some of the existing literature relating to DKD, which is the area of focus of this doctoral thesis.

2.2 Diabetic kidney disease in type 1 diabetes

2.2.1 Clinical staging and nomenclature

DKD, also termed diabetic nephropathy, is characterized by a clinical triad: abnormally increased urinary leakage of the protein albumin (albuminuria), elevated blood pressure, and gradual loss of kidney function. It can also occur without albuminuria, but whether or not this phenotype affects a considerable share of individuals with type 1 diabetes remains debated [54–56]. Figure 1 presents the clinical course typical to DKD in type 1 diabetes.

Albuminuria Proteinuria was initially screened with dipstick tests that could only spot large quantities of proteins excreted in the urine, typically in the range of several grams per day. The first immunoassay method for detecting albumin was described in 1963 [57]. However, it was not until the 1980s that minutely raised albumin excretion rate (AER), coined *microalbuminuria* by Viberti *et al.*, was distinguished as the first clinical stage of DKD [17]. To date, the Kidney Disease: Improving Global Outcomes (KDIGO) position statement recommends using the terms *moderately increased albuminuria* or *moderate albuminuria* instead of microalbuminuria. The diagnosis of moderate albuminuria and the more advanced stage *severely increased albuminuria* or *severe albuminuria* (formerly *macroalbuminuria*) is based on international

reference limits in two of three consecutive 24-hour, timed overnight, or spot urine specimens [58].

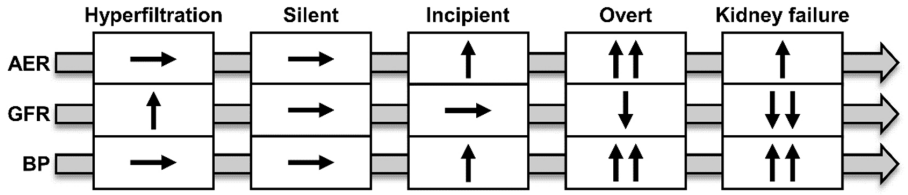


Figure 1 **The conventional five-step clinical course of diabetic kidney disease in type 1 diabetes.** →, normal; ↑, increased; ↑↑ severely increased; ↓, decreased; ↓↓, severely decreased. In diabetic kidney disease, the albumin excretion rate (AER) rises gradually until the stage of kidney failure, during which it declines due to the low kidney output. The initial changes in AER typically occur before the glomerular filtration rate (GFR) starts to decrease. The blood pressure (BP) typically begins to increase simultaneously with the occurrence of moderate albuminuria, and hypertension is a frequent feature of severe albuminuria and kidney failure.

Originally, 24-hour urine collections were the gold standard in clinical care for albuminuria screening. However, because the 24-hour urine collections were considered rather difficult for the patients leading to potential inaccuracies in the collections (time and volume), the golden standard shifted towards timed overnight urine collections. Even though overnight urine collections are easier to perform than the 24-hour collections, they were still considered cumbersome. Consequently, when albuminuria testing became a routine part of the care of individuals with type 2 diabetes, the American Diabetes Association (ADA) recommended the less cumbersome albumin-creatinine-ratios (ACRs) from spot urine collections as the new golden standard for albuminuria screening in diabetes [59]. The ACR, or some other validated measure of albuminuria, should be monitored at least annually in all patients with type 1 diabetes and a disease duration of at least five years [59]. Studies comparing different albuminuria determination methods are few, yet the existing ones generally present positive results; for instance, in a large type 2 diabetes study sample from the PREVEND (*Prevention of Renal and Vascular End-Stage Disease*) and RENAAL (*Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan*) trials, the results indicated high agreement between early morning ACR and 24-hour AER regarding the categorization of albuminuria [60].

Kidney function Glomerular filtration rate (GFR) is widely regarded as the best index for kidney function. GFR cannot be determined directly, but the clearance of exogenous filtration markers can be measured (measured GFR, or mGFR) and used as a highly accurate surrogate marker. The gold standard is to measure the urinary clearance of intravenously infused inulin, but more straightforward methods using iohexol or iothalamate, for example, have been

developed [61]. Even so, mGFR is mainly limited to specialized facilities, such as research settings. The clinical practice guidelines recommend using creatinine-based estimated GFR (eGFR) as the primary index for kidney function [62]. The advantage of eGFR over mGFR is that it does not require the measurement of exogenous filtration markers but is instead based on endogenous indicators. Several GFR-estimating equations have been developed and validated throughout the years, among them the Cockcroft-Gault formula for creatinine clearance in 1976 [63] and the formula derived from the Modification of Diet in Renal Disease Study in 1999 [64]; however, the most precise creatinine-based formula [61] is the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula from 2009 [65]. Confirmatory tests are endorsed when creatinine-based eGFR is likely to be inaccurate, for instance, in individuals with muscle-wasting or very high muscle mass. GFR can also be estimated in these individuals using serum cystatin C, which is less affected by the musculature (but is, in contrast, more influenced by adiposity, inflammation, smoking, thyroid dysfunction, and large doses of glucocorticoids [66,67]), or a combination of serum creatinine and cystatin C. The most recent eGFR formula, incorporating both creatinine and cystatin C, was presented in 2021 (Inker *et al.*) [68]. Opposite to, for instance, the CKD-EPI formula, the most recent formula does not consider race in the calculations but gives rise to smaller differences between Black and non-Black participants.

Based on estimated or measured GFR, kidney function can be categorized into the following stages; normal or high (G1, ≥90 mL/min per 1.73 m^2), mildly decreased (G2, 60-89), mildly to moderately decreased (G3a, 45-59); moderately to severely decreased (G3b, 30-44); severely decreased (G4, 15-29); and kidney failure (G5, <15) [62].

Glomerular hyperfiltration, meaning GFR elevated to the supraphysiological range, is observed in up to two of three individuals with type 1 diabetes – especially early in the course of the disease and in those with poor glycemic control. Hyperfiltration is also noted more often in individuals who are obese. No general definition of hyperfiltration has been agreed upon; thus, thresholds ranging between 120 and 140 mL/min per 1.73 m^2 appear in the academic literature. [69]

Chronic kidney disease The KDIGO defines chronic kidney disease (CKD) as "abnormalities of kidney structure or function, present for >3 months, with implications for health." CKD can thus be defined either as a decreased GFR <60 mL/min per 1.73 m^2 or as the presence of at least one marker of kidney damage, including moderate or severe albuminuria, abnormalities detected by histology, or history of kidney transplantation, among other traits [62]. Therefore, it is currently advised that CKD should be classified both based on the underlying cause, the appropriate GFR category as previously presented (G1 to G5), and the stage of albuminuria [62].

Kidney failure The most advanced stage of CKD (GFR <15 mL/min per 1.73 m^2) is, to date, referred to as kidney failure. Previous names, yet not advocated anymore, include end-stage renal disease, end-stage kidney disease, renal failure, and kidney insufficiency, among others. Possible treatment modalities for kidney failure are dialysis and kidney transplantation. These are together referred to as kidney replacement therapy.

2.2.2 Epidemiology

Early studies The first epidemiological reports on DKD in type 1 diabetes date back to the 1970s and 1980s. Two early studies from the Steno Memorial Hospital in Denmark and the Joslin Clinic in the United States, assessing individuals with the onset of type 1 diabetes during the first half of the 20th century, found that the cumulative incidence of persistent proteinuria was 25 to 35% after 25 years of diabetes and 35 to 45% after 40 years [14,15]. The Danish study by Andersen *et al.* found that proteinuria occurred in two peaks: the first and larger after 16 years of diabetes and the second after 32 years [14]. In the study from the US, Krolewski *et al.* found that the incidence of proteinuria peaked 10-14 years after the debut of diabetes, whereas the existence of a second incidence peak could not be replicated [15]. Likewise, in another study from the Steno Memorial Hospital but with somewhat different inclusion criteria, only one incidence peak was observed (at 13-18 years of diabetes duration) [70].

Based on this incidence rate pattern, the early studies speculated that only 45-50% of individuals with type 1 diabetes would be predestined to develop DKD [14]. The characteristic natural history with a drop in the incidence rate after 15 to 20 years of diabetes was thought to reflect a genetic predisposition to kidney complications, because if proteinuria developed exclusively due to the cumulative glycemic burden, the incidence would continue its steady rise until the majority of individuals would be affected [71]. The prognosis looked brighter if a diabetes duration of 35 years had been attained in the Danish cohort, because only 4% of the studied individuals developed proteinuria after this milestone [14].

The study by Krolewski *et al.* was also the first to evaluate changes in the incidence of DKD over time. The study revealed a declining trend: The risk of persistent proteinuria had approximately halved between the 1930s and 1940s-1950s diagnosis cohorts [15].

The first studies relating to moderate albuminuria (then called microalbuminuria) were also published during the early 1980s. Three landmark studies projected a coarse prognosis for individuals with microalbuminuria: During 6-14 years of follow-up, 63-88% progressed to persistent proteinuria [17–19]. Even though the study participants with initial moderate albuminuria were few (altogether 30 individuals), the findings led

to the assumption that progression is practically inexorable when albuminuria occurs.

Moreover, the prognosis continued to be poor if proteinuria developed, because the majority progressed to kidney failure. The median time between the appearance of proteinuria and the initiation of kidney replacement therapy, as evaluated in 1985, was ten years. The cumulative progression rate from proteinuria to kidney failure was 25% at six years and 75% at 15 years [15].

Moderate albuminuria The studies that have longitudinally assessed the occurrence of moderate albuminuria in type 1 diabetes are compiled in Table 1. The summary shows that most existing studies are based on a single-center setting, and, of note, no studies have evaluated the incidence of moderate albuminuria in a population-based type 1 diabetes cohort.

It is also noteworthy that only three studies have followed individuals from their diabetes onset onwards – thus, selection bias arising from only including individuals with normal AER but a diabetes duration of up to 20 years at baseline may be an issue for the others. Amin [72] and Goñi [73] *et al.* recruited participants with newly diagnosed type 1 diabetes to their respective studies; however, with mean observation times of only ten years, many incident cases of moderate albuminuria may have been missed. The study with the longest follow-up so far of individuals from their diabetes diagnosis (1979-84) onwards was published by Hovind *et al.* in 2004 [74]. In the study, 222 participants were annually checked for the presence of albuminuria over ~18 years. The cumulative incidence of moderate albuminuria was 34%.

Unfortunately, no study has been able to show a drop in the occurrence of moderate albuminuria – yet, the low number of reports on temporal trends might be to blame. The Pittsburgh Epidemiology of Diabetes Complications (EDC) Study showed that the cumulative incidence of moderate albuminuria was not smaller in the 1965-80 than in the 1950-64 diagnosis cohort [75]. The incidence of moderate albuminuria similarly remained unchanged in a UK-based cohort of children diagnosed with type 1 diabetes during 1986-96, despite some improvements in glycaemic control [76].

Severe albuminuria Some, although limited, epidemiological analyses of severely increased albuminuria have been published after the early reports outlined earlier. The DKD reference limits vary considerably in these and include definitions beyond current recommendations, potentially affecting the results. Moreover, population-based studies of sufficient size to evaluate the epidemiology of severe albuminuria are rare. Importantly, as the diabetes diagnoses are restricted to the era before the 1990s, updated information to track the contemporary natural history of established DKD is needed.

Table 1 Observational studies (no interventions) assessing the incidence of moderate albuminuria in type 1 diabetes. At least two albuminuria measurements during the observation period were required for inclusion, as well as defined criteria for moderate albuminuria (*i.e.*, a lower and an upper reference limit). If several papers had been published from the same cohort, the one with the longest follow-up time was selected to the table. *For the Microalbuminuria Collaborative Study Group. †Number at risk at different time points was not reported. ‡For the Pittsburgh Epidemiology of Diabetes Complications Study. SC, single-center; MC, multi-center; N, national; MN, multi-national; yr, year(s); mo, month(s); MA, moderate albuminuria. Values inside parentheses denote 95% confidence intervals.

First author, year	Design	Baseline year(s)	Baseline diabetes duration	Follow-up time	Measurement frequency/quantity	No. MA/ No. subjects	Incidence rate or cumulative incidence (if reported)
Mathiesen, 1990 [77]	SC	1982-83	Mean 16-17 yr	5 yr	Every 4 mo	15/205	NA
Marshall*, 1999 [78]	MC, N	1984-86	Mean 12-18 yr	Median 7 yr	Every 6 mo	14/148	7-yr cumulative incidence 11% (6.36-16.94)
Rossing, 2002 [79]	SC	1984	Mean 20 yr	Median 9 yr	At least annually	134/537	NA
Esmatjes, 2002 [80]	MC, N	1995	Mean 14 yr	Mean 4.3 yr	Every 3-5 mo	110/947	Annual incidence 2.7% (2.2–3.2)
Hovind, 2004 [74]	SC	1979-84	Newly diagnosed	Median 18.0 yr	At least annually	79/222	18-yr cumulative incidence 33.6% (27.2-40.0)
Steinke, 2005 [81]	MC, MN	NA	Mean 8.0 yr	5 yr	Every 3 mo	22/170	NA
Stone, 2006 [82]	SC	1989-04	Median 6.5 yr	Median 6.2 yr	Median 4 samples per patient	59/767	4.6 per 1,000 patient-years
Amin, 2008 [72]	SC	1986-97	Recruited within 3 mo of diagnosis	Mean 9.8 yr	Annually	135/527	Cumulative incidences: 10-yr: 25.7% (21.3-30.1) 19-yr: 50.7% (40.5-60.9)

Cobas, 2011 [83]	SC	1991	Median 3 yr	Median 6.8 yr	≥2 follow-up samples per patient	50/122	6.8/100 people per year (5.04-8.95)
Romero-Aroca, 2012 [84]	SC	1991	Mean 13.1 yr	20 yr	Every 6 mo	47/110	NA
Galler, 2012 [85]	MC, MN	1995-05	Mean 4.6 yr	5 yr	≥2 urine analyses per year	110/516	NA
Goñi, 2016 [73]	SC	1991-08	Newly diagnosed	Mean 10.1 yr	At least annually	53/716†	Cumulative incidences: 5-yr: 2.5% (1.3-3.6) 10-yr: 6.1% (4.0-9.1) 15-yr: 10.6% (7.4-13.6)
Costacou‡, 2018 [75]	SC	1950-80	Recruited within 1 yr of diagnosis	Up to 50 yrs	Every 2 yr	Diagnosis cohort 1950-64: 176/224 at 50 yr of follow-up Diagnosis cohort 1965-80: 188/230 at 40 yr of follow-up	Cumulative incidences in 1950-64 cohort: 30-yr: 65.2% 40-yr: 79.0% 50-yr: 88.0% Cumulative incidences in 1965-80 cohort: 20-yr: 54.7% 30-yr: 70.0% 40-yr: 81.7%

Some years after the early study by Andersen and colleagues [14], Koefoed-Enevoldsen *et al.* reported that the 25-year cumulative incidence of proteinuria had diminished from 41 to 27% between the 1933-42 and 1954-62 diabetes diagnosis cohorts in Denmark [16]. Nordwall *et al.* further showed that the 25-year cumulative incidence had dropped beyond this between 1961-65 and 1971-75 in the Swedish Linköping Study, from 30 to 13% [86]. Another more recent Danish report did not observe a decrease in the incidence among individuals with the onset of type 1 diabetes in 1956-79 when the observation period was restricted to 1991 [87], whereas a follow-up analysis revealed a downward trend [88]. However, the researchers could only see a difference in the severe albuminuria risk between 1965-74 (the two earliest calendar-year cohorts combined) and 1979-84 (the most recent calendar-year cohort). The 20-year cumulative incidence was 30% in the earlies cohorts, while it was 14% in the most recent one [88].

Still, not all studies have been able to show a decreasing trend in the incidence of severe albuminuria with increasing calendar year of diabetes onset. Two calendar-year cohorts were compared in the Pittsburgh EDC cohort: individuals with type 1 diabetes diagnosed during 1950-64 (n=201) *vs.* during 1965-80 (n=388). The proportion afflicted by albuminuria was no different between the cohorts, with ~40% of the participants advancing to severe albuminuria during the first 30 years of diabetes and nearly 60% during the first 40 years. After 50 years of diabetes duration in the earlier cohort, the cumulative incidence of severe albuminuria had already surpassed 70%. [75]

Regression of albuminuria The view of a committed course towards albuminuria progression was challenged for the first time over a decade after the early papers by Viberti [17], Parving [18], and Mogensen [19] appeared. The concept of albuminuria regression is well-recognized to date, although no generally accepted definition of it exists. Classifications based both on categorical cut-offs and changes in continuous AER measurements (*e.g.*, 30 or 50% reduction) appear in the academic literature.

In 1992, Forsblom *et al.* published a ground-breaking prospective study that questioned moderate albuminuria as a predictor of overt DKD (progression rate only 28% over 10 years) and even observed regression from severe to moderate albuminuria (regression rate 14%) [89]. Another study that rightfully earned seminal status and has significantly contributed to the expertise we have in DKD today was published by Perkins *et al.* from the Joslin clinic in 2003. The study, in contrast to what was known from before, discovered a six-year cumulative regression rate of 58% from moderate to normal albuminuria. The nearly 400 study participants had a mean diabetes duration close to 20 years but varying durations of moderate albuminuria (the study population comprised both an incidence and a prevalence cohort) at baseline [90].

However, regression, like progression, is not a static state, and AER levels tend to fluctuate both upwards and downwards, especially early in the course

of diabetes and diabetic albuminuria. This has been shown, for instance, by Amin *et al.*, who followed subjects with short-duration type 1 diabetes and normal AER at baseline for five years [72]. Of those whose albuminuria advanced to the moderately increased range, ~50% reverted to normal AER during successive years; however, when the observation period was extended past the regression, a re-appearance of albuminuria in every fourth regressor was further revealed. The relapse rate after moderate albuminuria regression was 25% in the Diabetes Control and Complications Trial (DCCT) [91]. In the study by Hovind *et al.* [74], although 35% of the individuals with incident moderate albuminuria regressed to normal AER, over half relapsed to albuminuria during the observation time (median 7.5 years).

It is noteworthy that regression of severe albuminuria has also been encountered, though it was long considered an irreversible DKD stage – *the point of no return*. Regression rates vary between 7 and 52% in the literature and have mostly, yet not entirely, been observed under aggressive antihypertensive treatment [89,92,93]. Aggressive blood pressure lowering has also been shown to result in moderate albuminuria regression [94,95], although spontaneous regression from moderate albuminuria appears to be more common than from the more severe stage.

A noticeable knowledge gap exists regarding regression of albuminuria, because there is a limited and inconsistent understanding of its consequences in type 1 diabetes. It has been shown in type 2 diabetes that a 50% reduction of albuminuria initially in the moderately increased range translates into a significantly lower burden of death from/hospitalization for renal and cardiovascular events [96]. However, although remission of nephrotic-range albuminuria by aggressive antihypertensive treatment improved the survival in a small type 1 diabetes cohort [97], regression of moderate albuminuria was not succeeded by cardiovascular risk reduction in the DCCT [91].

Kidney failure Finland commenced dialysis treatment in 1961, and the first kidney transplantation was performed three years later. CKD due to diabetes was included as a treatment indication in 1973. Active treatment of uremia increased rapidly after this, and more organ transplantations per capita were performed in Finland in 1976 than in any other European country [98]. The need for active uremia treatment has been met in the whole country from the early 1970s onwards [99].

Incident events of kidney failure have been tracked since 1964 through the Finnish National Registry for Kidney Diseases. Recent results retrieved from the registry are optimistic: A dramatic decrease in the need for kidney replacement therapy due to type 1 diabetes has occurred during the past five decades in Finland. The most recent report showed that the 20-year cumulative incidence of kidney failure for Finns with type 1 diabetes diagnosed in 1980-2011 was 1.3%, and the 30-year cumulative incidence was 4.4% [100]. The cumulative incidence of kidney failure has gradually decreased over a

longer period of time, yet the gap between the 1965-79 diagnosis cohort and the more recent ones is the most pronounced [100].

Nevertheless, type 1 diabetes remains a notable cause of kidney failure in Finland. It was the second most common underlying diagnosis among individuals who entered kidney replacement therapy in 2020, surpassed only by type 2 diabetes [101].

The updated Finnish estimates of the kidney-failure risk over time are almost analogous to results from Norwegian [102] and Swedish [103] population-based surveys and a single-center study from Japan [104]. However, substantially higher cumulative incidences have also been described; for instance, in the Pittsburgh EDC cohort, the cumulative incidence of kidney failure was 15% after 30 years in the later diagnosis cohort (diabetes diagnosis 1965-80), and up to 60% after 50 years of diabetes in the earlier diagnosis cohort (1950-64) [75]. Most studies have agreed on a decreased need for kidney replacement therapy due to complications of type 1 diabetes over time [75,100,104,105]; however, contradictory results have also appeared. For instance, in an analysis of kidney failure during 1991-2000 in 10 European centers combined, the incidence development actually showed a small rise for type 1 diabetes in the combined dataset [106]. Furthermore, in the Norwegian population-based study, the incidence of kidney failure showed no signs of decline between the 1973-82 and 1989-2012 observation cohorts, but this might be attributable to the very low incidence throughout the observation period [102].

2.2.3 Pathogenesis

The pathogenesis behind the initiation and progression of DKD is known to be multifactorial but remains incompletely understood. Describing it with brevity is challenging because it consists of several chains of events occurring in parallel. Figure 2 presents an attempt.

Glucotoxicity is the initiator of the sequence. More specifically, as originally stipulated by Professor Brownlee, the cascade is initiated by hyperglycemia-induced overproduction of reactive oxygen species (ROS) by the mitochondrial electron-transport chain. The superoxides are thought to decrease the activity of a key glycolytic enzyme (glyceraldehyde-3 phosphate dehydrogenase, or GAPDH), leading to increased levels of glycolytic intermediates that are upstream of the enzyme and, thereby, to the activation of four damaging pathways. [107]

The four pathways are as follows: First, hyperglycemia leads to increased intracellular production of precursors for advanced glycation end-products (*i.e.*, glycated proteins or lipoproteins), which can then modify intracellular proteins, extracellular matrix components, or induce production of ROS by binding to specific receptors on other cells. Second, hyperglycemia upregulates the so-called polyol pathway, leading to intracellular sorbitol

accumulation (and thereby osmotic stress) and NADPH depletion (and, thereby, ROS production). Third, hyperglycemia increases the production of diacylglycerol, which activates protein kinase C, which plays an essential role in several signal transduction cascades affecting vascular permeability and angiogenesis (*e.g*, by decreasing the production of endothelial nitric oxide synthase). Fourth, hyperglycemia leads to upturned flux through the so-called hexosamine pathway, which influences gene expression and protein function in many ways. The downstream effects of these four damaging pathways are abundant; most are mediated through the release of pro-inflammatory cytokines and growth factors, activation of transcription factors, or increased procoagulant activity. [107]

Metabolic alterations in the extracellular matrix regulation lead to abnormal production of extracellular matrix proteins and decreased expression of matrix metalloproteinases [108]. As a result, the first characteristic histological features of DKD – glomerular basement membrane thickening and following mesangial expansion – emerge [109]. However, hemodynamic processes also contribute to the mesangial expansion. The glomerular filtration of glucose increases in the presence of hyperglycemia, subsequently upregulating the expression of tubular sodium-glucose cotransport proteins. The delivery of sodium chloride to the macula densa is decreased due to raised reabsorption of sodium in the proximal tubule, resulting in afferent arteriole vasodilation via the intrinsic tubuloglomerular feedback trail. It also stimulates the activation of the renin-angiotensin-aldosterone system (RAAS), leading to efferent arteriole vasoconstriction due to the upsurge in angiotensin II production. Of note, medicines that target the RAAS system are used in the treatment of DKD (section 2.2.5). The metabolic and hemodynamic factors together amplify the renal perfusion and increase the intraglomerular pressure, exposing the glomeruli to shear stress [110].

The third glomerular characteristic of DKD is the appearance of focal (in less than 50% of the glomeruli) nodular mesangial lesions with an acellular hyaline/matrix core, termed Kimmelstiel-Wilson lesions. Global (in more than 50% of the glomeruli) sclerosis marks the fourth and most advanced histological stage of DKD [109]. The grading of diabetic kidney lesions relies on the glomerular features, but the tubular system is no bystander in DKD. Many early hyperglycemia-induced tubular pathologies, especially in the proximal tubules, can be detected [111].

Hyperfiltration in the diabetic kidney is likely due to a combination of transient structural changes (nephromegaly resulting from hyperglycemia-induced cytokines and growth factors), vascular factors (imbalance of vasoactive humoral factors, such as nitric oxide, angiotensin II, and ROS), and tubular factors (the tubuloglomerular feedback system) [69]. Many researchers have theorized that hyperfiltration contributes to the initiation and progression of clinical DKD [112]; however, available data are contradictory [113,114]. The clinical manifestations of DKD start to gradually evolve (Figure 2) after the potential hyperfiltration and the “silent” stage

(Figure 1). However, as reviewed earlier, not all individuals with type 1 diabetes will ever develop DKD. Many risk factors for DKD have been identified, some of which will be reviewed in the next section.

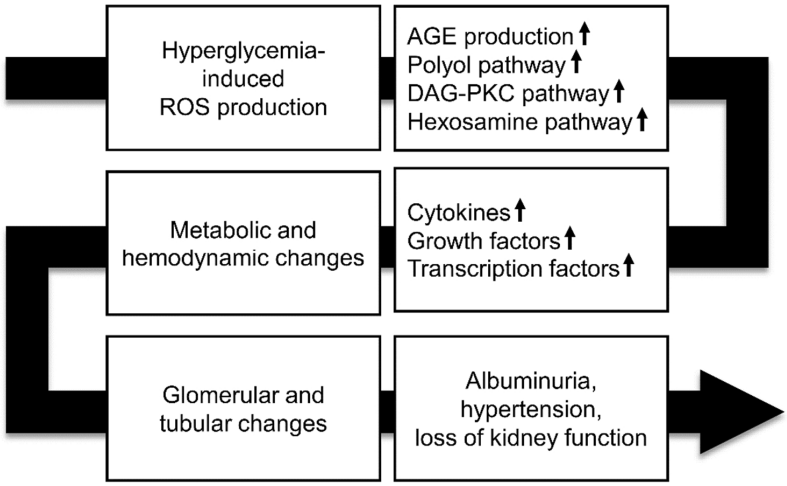


Figure 2 **The key and events leading to the development of diabetic kidney disease.** ROS, reactive oxygen species; AGE, advanced glycation end-product; DAG-PKC, diacylglycerol-protein kinase C.

2.2.4 Risk factors

Sex and age at diabetes onset A young age (0-4 years) at the onset of diabetes has been associated in many studies with the lowest risk of DKD [75,115,116]. However, whether sex is a risk factor for DKD is not without controversy. Most studies agree on female protection [74,117,118], while others have found no sex difference in the risk [79,116] or even a female preponderance [119] for DKD. This inconsistency is probably a result of several factors, such as differences in study designs and studied cohorts [120]. However, findings from the FinnDiane Study [121] and Swedish nationwide registers [103] suggest that the impact of sex is dependent on the diabetes onset age, which unadjusted could contribute to the discrepancy in results. No difference in the risk of kidney failure was observed in the FinnDiane cohort between women and men with diabetes onset at 0-9 years, whereas among individuals with diabetes diagnosed at 10-14 and ≥15 years, men exhibited a 1.8 and 1.9-fold increased risk compared to women, respectively [121]. The occurrence of kidney failure in Sweden was similar for men and women with diabetes onset <20 years, but if the diabetes diagnosis had been set at 20-34 years, a risk excess was found

among the men (hazard ratio [HR] 2.3) [103]. These findings imply a role for sex hormones, which remains to be explored in detail.

Familial factors and genetic predisposition A Finnish longitudinal population-based study covering more than 500 sib pairs with type 1 diabetes revealed that the presence of DKD in one sibling doubles the risk in the other [122]. Analogous results have been drawn from other smaller cohorts [123,124], whereas the parent-offspring concordance rate has received less attention. Nevertheless, other parental factors, such as parental insulin resistance [125,126], diabetes [127,128], and hypertension [125,127,128] appear to confer a heightened DKD risk. The narrow-sense heritability – meaning the proportion of phenotypic variance explained by additive genetic variation (in this case based on genome-wide single nucleotide polymorphisms) – is ~35% when DKD is defined as moderate albuminuria, severe albuminuria, or kidney failure [129]. The narrow-sense heritability estimate is as high as 59% when eGFR <60 mL/min per 1.73 m^2 is added as a diagnostic criterion [129]. However, the quest for candidate genes for DKD has been complicated, and the genetic background remains unsuccessfully unraveled. Genome-wide association studies have so far identified six potential susceptibility loci for DKD in type 1 diabetes [130]. However, the method is suitable for detecting common variants, whereas low-frequency and rare variants mostly remain overlooked. Future exome-wide and genome-wide sequencing studies are thus expected to bring new information about the genetics underlying DKD and the other diabetic complications.

Suboptimal glycemic control The pivotal role of hyperglycemia as a risk factor for DKD stems from the ground-breaking Diabetes Control and Complications Trial (DCCT). The DCCT was a multicenter randomized controlled trial comprising over 1,400 individuals assigned to either the conventional therapy (one or two daily insulin injections) or intensified therapy regimen. The intensive therapy aimed to achieve near-normal glucose levels with insulin administered at least three times daily and insulin dosage adjusted according to self-monitored blood glucose concentrations. Intensified treatment resulted in significantly improved glycemia and a markedly decreased risk of DKD during the mean follow-up of 6.5 years: The mean adjusted risk of severe albuminuria was reduced by 54% and moderate albuminuria by 39% [131]. The observational Epidemiology of Diabetes Interventions Study (EDIC) was founded to follow the durability of the DCCT outcomes after the completion of the DCCT. Interestingly, a prolonged effect of the intensive therapy on the occurrence of DKD and other vascular complications was observed in the EDIC study setting, even though the achieved difference in HbA_{1c} between treatment arms began to narrow after the DCCT closeout [132]. This phenomenon has been termed the metabolic memory. The risk of impaired eGFR (<60 mL/min per 1.73 m^2) was 50% lower in those who had received intensive therapy during the combined DCCT/EDIC follow-up [132].

New metrics of glucose exposure that go beyond HbA_{1c} concentration have emerged as factors possibly influencing the DKD risk after the DCCT. Intraindividual visit-to-visit HbA_{1c} variability is one such factor [133,134]. Recent reports also argue that the time in range of optimal glycemia, obtained from continuous glucose monitoring technology, strongly predicts incident moderate albuminuria in type 1 diabetes [135]. Hence, it appears that even in the case of optimal average blood glucose, transient spikes of hyperglycemia may be sufficient to provoke target organ damage.

Hypertension Hypertension is prevalent in type 1 diabetes: Among the adult patients enrolled in the Coronary Artery Calcification in Type 1 Diabetes cohort (mean age 37 years) in the early 2000s, hypertension was present in 43% but only in 15% of the nondiabetic control population [136]. Even though hypertension also occurs frequently in the absence of DKD [137], it is highly associated with albuminuria and abnormal kidney function in type 1 diabetes [138]. Several pathophysiological mechanisms by which DKD increases blood pressure have been untangled; for instance, the amplified renal sodium reabsorption and the following volume expansion, as well as the RAAS activation and subsequent peripheral vasoconstriction [138]. However, hypertension is regarded not only as a central clinical feature of DKD but also as a potent risk factor for its progression [74,139]. This goes in line with the fact that many clinical trials have revealed renoprotection of effective antihypertensive treatment (section 2.2.5).

Dyslipidemia Several studies have reported plasma lipid abnormalities in individuals with DKD, especially at advanced stages of disease. Some lipid abnormalities have also been associated with a raised risk of DKD progression. Of the conventional lipid profile in the EURODIAB cohort, high low-density lipoprotein (LDL) cholesterol, high triglycerides, and low high-density lipoprotein (HDL) cholesterol predicted the development of incident moderate albuminuria independent of diabetes duration, HbA_{1c}, and baseline AER [140]. No serum lipid species remained significant after adjustment when the progression from moderate to severe albuminuria was assessed in the population, although a univariately significant difference in triglyceride concentration was initially seen (higher among progressors) [141]. High total cholesterol, LDL-cholesterol, and triglycerides predicted the progression of moderate to severe albuminuria in unadjusted analyses in the DCCT/EDIC cohort [142]. The triglyceride concentration was a strong predictor of the progression from normal AER to moderate albuminuria in the FinnDiane cohort, whereas only total cholesterol was significant in the multivariable model at the step from severe albuminuria to kidney failure [20].

Conventional lipid species have also appeared as predictors of the regression of albuminuria in some studies. Perkins *et al.* from the Joslin Diabetes Center discovered an association between lower baseline triglycerides, total cholesterol, and the regression of moderate albuminuria

[90]. The same parameters and low LDL-cholesterol were linked to regression of severe albuminuria in the DCCT [142]. However, in the EURODIAB study, no lipid parameter from the conventional lipid profile was associated with albuminuria stage regression in multivariable analyses [141].

Nuclear magnetic resonance spectroscopy analyses have provided further understanding of associations between lipoprotein particle sizes and compositions with DKD outcomes, although the existing evidence is conflicting. In a subgroup analysis of the FinnDiane cohort, large and extra-large very-low density lipoprotein (VLDL) particle subclasses were linked to the highest odds for the development of severe albuminuria [143]. The analyses additionally discovered a triglyceride-cholesterol imbalance among the lipoprotein classes in individuals with DKD, particularly enrichment of triglycerides and/or cholesterol in VLDL subclasses in individuals at high risk of albuminuria progression [144]. Only concentrations of the medium and small VLDL subclasses were elevated in DKD in female participants of the DCCT trial, whereas all VLDL subclasses, in addition to intermediate-density lipoprotein (IDL) and small (noncardioprotective) HDL subclasses, were increased in men with DKD [145]. However, in the Pittsburgh EDC study, there was no difference either in the VLDL or HDL subclass distribution or in the concentration of IDL lipoprotein particles between those who did and did not progress to overt nephropathy. However, the progressors were distinguished by diminished LDL particle size [146].

Beyond lipid plasma concentrations, results from type 2 diabetes have linked amplified intraindividual lipid variability with an increased DKD risk. More specifically, high triglyceride variability has been proposed to predict incident moderate albuminuria [147] and high HDL-cholesterol variability the progression of DKD [148]. Lipid variability in relation to DKD in type 1 diabetes has, however, not been studied.

Other risk factors Several risk factors for DKD relating to lifestyle have been identified, such as sedentary behavior [149] and smoking [150,151]. There is genetic evidence for a causal relationship between obesity and DKD in type 1 diabetes [152], and insulin resistance appears to precede the development of albuminuria [153] and kidney function decline [154]. In relation to this, the prevalence of metabolic syndrome increases with the severity of DKD [154].

2.2.5 Treatment strategies

As will be appraised below, DKD is a main determinant of increased cardiovascular risk and shortened life expectancy in type 1 diabetes. Hence, the care recommendations strive not only to prevent DKD or halt its progression but also to reduce the concomitant cardiovascular and premature mortality risk. Thus, the treatment strategies are not specific only to DKD. The emphasis is on glycemic and blood pressure control.

In diabetes in general, the ADA recommends an achievement goal of near-normoglycemia [59]; hence, HbA_{1c} <53 mmol/mol (<7%) should be targeted in nonpregnant adults with diabetes [155]. The microvascular risk also continues to drop under this threshold, even though the absolute risk reduction becomes smaller. Based on the physician's judgment, a more stringent HbA_{1c} target can therefore be set in some individuals; however, a potential challenge in the context of DKD is the increased hazard of hypoglycemia. This is due to many factors, such as reduced renal insulin clearance and the diminished renal gluconeogenesis [156]. Insulin degradation may also be decreased in peripheral cells in a uremic milieu due to the accumulating uremic toxins [156,157].

Multiple daily injections of insulin or continuous subcutaneous insulin infusion form the cornerstones of glycemic control in type 1 diabetes [158]. The availability of therapeutic options is broader in type 2 diabetes, and in view of DKD, the sodium-glucose cotransporter 2 (SGLT2) inhibitors are of particular interest. The SGLT2 proteins reside in the epithelial cells of the proximal convoluted tubules and are responsible for ~90% of the filtered glucose reabsorption. Selective inhibition of these transporters increases glucosuria, translating into an effective and hypoglycemia-safe reduction of blood glucose concentration [159]. SGLT2 inhibition confers great cardiovascular benefit, particularly regarding hospitalization for heart failure [160]. Additionally, as originally discovered by the CREDENCE (*Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation*) trial in 2019, the renal benefits are also outstanding. The trial established a 34% decline in the risk of kidney failure, doubling of serum creatinine, or renal death compared to placebo in individuals with type 2 diabetes and albuminuric CKD [161]. However, no class of drugs comes without possible adverse effects: With SGLT2 inhibitor therapy, those with low or no intrinsic insulin production (*e.g.,* individuals with type 1 diabetes) are especially predisposed to develop (euglycemic) diabetic ketoacidosis. Therefore, potential off-label treatment with SGLT2 inhibitors is recommended to be undertaken with caution, particularly in patients with type 1 diabetes [162].

Blood pressure should be measured at every routine clinical visit [163]. The ADA recommends considering stricter blood pressure targets (such as <130/80 mmHg) for individuals with diabetes who are at high risk for renal or cardiovascular outcomes, for instance those with CKD [163]. The KDIGO's care guidelines, updated in 2021, suggest a target systolic blood pressure of <120 mmHg, if tolerated, in those with CKD [164]. In contrast, the anticipated benefits *vs.* risks of intensive blood pressure lowering in individuals with severely decreased kidney function or kidney failure remain uncertain [163,164].

The preferred first-line antihypertensive agents among individuals with diabetes, hypertension, and CKD are angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) [59]. The use of a RAAS

blocker is also recommended in normotensive individuals with moderate albuminuria. However, no evidence for salutary effects of primary prevention with a RAAS blocker in normotensive and normoalbuminuric individuals with diabetes exist [59,165]. Selective mineralocorticoid receptor antagonism with the agent finerenone in addition to RAAS blockade has been shown to decrease urinary ACR levels in type 2 diabetes [166]. Additionally, it was recently revealed that finerenone lowers the risk of kidney disease progression in this patient group [167].

There is limited evidence concerning the effects of lipid-lowering medication on DKD outcomes in type 1 diabetes in contrast to antihypertensive treatment.

The SHARP (*Study of Heart and Renal Protection*) study was a randomized-controlled trial launched to investigate the vascular effects of LDL-cholesterol lowering by low-dose simvastatin combined with ezetimibe therapy in over 9,000 participants with CKD [168]. The proportion of type 1 diabetes was not stated, although the underlying cause of CKD was DKD in 15%. The trial demonstrated marked protection against major atherosclerotic events among the CKD individuals receiving a combination lipid-lowering treatment [169]. However, the LDL-cholesterol reduction (~1 mmol/L) that followed the intensified lipid therapy had no significant impact on the development of kidney failure or doubling of baseline serum creatinine during the nearly five-year follow-up [170]. Furthermore, there was no effect on the rate of eGFR decline [170]. Moreover, in a meta-analysis on randomized-controlled trials evaluating the effects of statin use in diabetes, no improvements in albuminuria were found in the type 1 diabetes subgroup (standardized mean difference -0.01, 95% -0.52 – 0.50) [171]. However, only two small-scale studies (total number of cases/controls 31/29) in type 1 diabetes were eligible, so conclusions could not be drawn. The effects in the type 2 diabetes group were, however, extremely encouraging –regarding both the improvement of albuminuria and eGFR change [171].

Therefore, for DKD care guidelines in type 1 diabetes, results have been generalized from type 2 diabetes – and, even more importantly, the well-defined cardiovascular benefits of normolipidemia overrule the lack of evidence for DKD. Consequently, strict lipid targets apply to individuals with type 1 diabetes and DKD [172].

2.3 Chronic complications beyond kidney disease

2.3.1 Diabetic retinopathy

Diabetic retinopathy was the sixth most common cause of moderate-to-severe vision worldwide in 2015 [173]. It also remains the second most common cause of blindness in working-age adults in modern times [174]. Signs of a declining

incidence have emerged [175,176], yet retinopathy remains the most prevalent long-term complication of diabetes, to some degree affecting nearly every individual who attains a type 1 diabetes duration of 25 years [177].

Diabetic retinal lesions are classified using the internationally validated ETDRS (*Early Treatment Diabetic Retinopathy Study*) scale into advancing stages of nonproliferative and proliferative disease [178]. Proliferative diabetic retinopathy is characterized by neovascularization, that is, the proliferation of new blood vessels appearing due to retinal ischemia. The new blood vessels are brittle and prone to leakage, often leading to vitreous hemorrhaging. Another form of diabetic retinopathy is macular edema, or diabetic maculopathy, which is distinguished by exudative fluid buildup at the macula, the area of the retina responsible for the central high-resolution vision.

Diabetic retinopathy and kidney disease share many risk factors, among them long diabetes duration, poor glycemic control, and hypertension [179]. Varying results have been obtained for the association between lipids and diabetic retinopathy. Hadjadj *et al.* showed in a French cohort of individuals with type 1 diabetes that hypertriglyceridemia predicts the development and progression of diabetic retinopathy, whereas there was no link between retinopathy and other components of a standard lipid profile [180]. This finding is in line with results from two large type 2 diabetes trials, FIELD (*Fenofibrate Intervention and Event Lowering in Diabetes*) [181] and ACCORD (*Action to Control Cardiovascular Risk in Diabetes*) [182], which revealed beneficial effects on diabetic retinopathy of fenofibrate treatment.

Regression of diabetic retinopathy has also been noted. Klein *et al.* reported in the *Wisconsin Epidemiologic Study of Diabetic Retinopathy* that the rate of cumulative improvement over 25 years was 18% [177].

Diabetic retinopathy tends to be asymptomatic during its first stages; thus, regular retinal screening is key for timely detection of the early lesions. Laser photocoagulation therapy is typically initiated at the stage of proliferative, but sometimes already at severe nonproliferative, diabetic retinopathy to reduce the risk of vision loss. Central-involved macular edema is treated with intravitreous injections of anti-vascular endothelial growth factor. [183]

2.3.2 Diabetic neuropathy

The third microvascular complication of diabetes is diabetic neuropathy. The prevalence estimates of diabetic neuropathy after decades of type 1 diabetes range between ~10 and 70% [184–186]. The large variation is a consequence of the highly variable clinical appearance and the ill-defined diagnosis of the disease.

Diabetic neuropathy is a neurogenerative disease that targets sensory, autonomic, and, to a lesser extent, motor axons. The predominant clinical manifestation is distal symmetric polyneuropathy, although peripheral diabetic neuropathy can also appear as radiculopathy or isolated

mononeuropathy. Autonomic neuropathy, defined as impairment of the sympathetic and parasympathetic nervous system, establishes as cardiac neuropathy, gastroparesis, cystopathy, or impotence, for example. [187]

The most typical symptom of distal symmetric polyneuropathy is burning pain in the feet [187]. However, up to half of the afflicted individuals remain asymptomatic [183]. This aggravates the diagnostics and is of particular note, because neuropathy is the main predictor of foot ulceration and subsequent lower-limb amputation in diabetes [188,189].

The risk factor profile for diabetic neuropathy has many similarities with those of the other microvascular diabetic complications. Long-term hyperglycemia is the number one risk factor and also the most important preventive treatment [186]. The association between lipids and diabetic neuropathy has been rather sparsely studied. However, findings from the EURODIAB study (multinational cohort, type 1 diabetes) showed that a raised baseline triglyceride level predicts incident distal symmetric polyneuropathy, irrespective of adjustment for the strong risk factors diabetes duration, HbA_{1c}, hypertension, and AER, whereas the other lipids were not independently associated with the endpoint [190].

2.3.3 Macrovascular complications

Macrovascular complications affect the large blood vessels of the circulatory system. The main manifestations of macrovascular diabetic complications are CAD, cerebrovascular disease, and peripheral artery disease. The common denominator for these conditions is atherosclerosis.

Atherosclerosis The buildup of lipid-loaded plaques (or atheromas) in the arterial wall is a complicated, multistep process. The process will be briefly delineated next.

The vascular endothelium plays a key part in regulating the vascular tone, structure, thrombogenesis, and fibrinolysis. Loss of the phenotypic homeostatic features of healthy endothelial cells, termed *endothelial dysfunction*, is considered an early step in atherogenesis and a mechanistic link between CVD risk factors and atherosclerosis [191]. Hyperglycemia has been shown to promote endothelial dysfunction, particularly via the overproduction of ROS [192].

Apolipoprotein B (apoB)-containing lipoproteins up to ~70 nm in diameter can cross the vascular endothelium. However, the details of this process and its controlling mechanisms remain poorly understood. LDL particles are the most abundant apoB-containing particles in plasma [193], and their causal role in the atherosclerotic process is well defined [194]. However, as will be discussed later in this thesis (section 2.4.2), the role of the other apoB-containing lipoprotein particles in atheroma formation has lately been the subject of attention [195].

The atherosclerotic plaque forms in the innermost layer of the tri-laminar vascular wall, the tunica intima [196]. Intimal retention of LDL occurs mainly via the interaction of positively charged apoB amino acyl residues with negatively charged arterial wall proteoglycans. Some molecules, such as apolipoprotein E (apoE) and apoC-III, increase the affinity for this interaction [194].

Once entrapped in the subendothelial space, LDL particles are susceptible to ROS-mediated or enzymatic oxidation, which in turn initiates an inflammatory response by triggering the upregulation of adhesion molecules and chemokines by endothelial cells. Monocytes are recruited to the intima and differentiate into macrophages. These cells, together with smooth muscle cells, engulf the oxidized LDL particles and form cholesterol-laden foam cells. [194]

After its development, the plaque continues to grow through further monocyte recruitment, smooth muscle cell migration, and lipid accumulation. The smooth muscle cells produce extracellular matrix molecules that entrap lipoproteins and, thereby, contribute to plaque progression. A necrotic core of cellular debris, covered by a fibrous cap, is gradually formed. Furthermore, an established feature of advanced atherosclerosis is intimal calcification, the severity of which has a prognostic value for CVD events and mortality. Possible complications of atherosclerotic plaques are ruptures and the production of thrombi due to erosion. These events lead up to “hard” CVD outcomes, including myocardial infarction and stroke, which are typically used as endpoints in clinical studies – such as those of this thesis. [196]

Atherosclerosis in type 1 diabetes CVD is not specific to type 1 diabetes, yet it is more common and occurs earlier than in individuals free of diabetes. Endothelial dysfunction and other subclinical macrovascular abnormalities are frequently encountered in children and adolescents with type 1 diabetes – even within the first decade of the disease [197–199]. Women with type 1 diabetes carry a relatively disproportionate burden of CVD, reflecting loss of the female protection from CVD that is seen in the general population [200]. Recent data have revealed an encouraging reduction in the long-term trends of CVD in type 1 diabetes during past decades; nevertheless, a considerable residual risk excess remains compared to the general population [201,202].

Some characteristics of atherosclerotic plaques attributable to diabetes have been distinguished. The atheromas of individuals with diabetes tend to be larger, more lipid-loaded, and more macrophage-occupied than those of individuals free of diabetes [203]. Additionally, individuals with type 1 diabetes tend to have more severe, diffuse, and distal CAD manifestations than individuals without diabetes [204,205], and these differences have already developed before the onset of symptoms [206]. Like CAD, peripheral artery disease also tends to have a more distal anatomical localization in patients with diabetes [207].

Atherosclerosis in type 1 diabetes with concomitant kidney disease Despite the risk also being heightened in the absence of albuminuria, DKD is one of the most important determinants of the incremental CVD risk in type 1 diabetes [208,209]. The risk rises stepwise together with the progression of DKD, as is demonstrated in Figure 3. The standardized incidence ratio for CAD – meaning the ratio between observed and expected cases – is 26.6 for the sexes combined at the kidney failure stage, but as high as 113 in women separately due to the lower absolute risk in women in the background population [210].

Coronary plaques in individuals with kidney failure (independent of the underlying kidney disease diagnosis) are distinguished by increased tunica media thickness and pronounced calcification [211]. However, in advanced kidney disease, the overall cardiovascular pathophysiology is different from the "traditional" one, likely due to the physiological and metabolic disturbances that develop secondary to the kidney disease. Accordingly, CVD events in patients on maintenance dialysis are largely driven by nonatherosclerotic events, such as heart failure, arrhythmias, and hemorrhagic stroke [212].

The mechanisms connecting DKD with CVD have not been fully discovered, although several theories exist. First, albuminuria is considered a marker not only of renal pathologies but also of systemic vascular injury, which means that individuals with albuminuria most likely have widespread damage of the vasculature. DKD and CVD also share several common risk factors, such as advanced age, hyperglycemia, hypertension, and dyslipidemia. However, the traditional risk factors do not fully explain the CVD burden conveyed by DKD, as has been shown by using the Framingham predictive instrument in individuals with CKD [213]. Thus, some other connections must exist. One plausible connection arises from the reduced nitric oxide bioavailability in kidney disease through the lowered expression and activity of endothelial nitric oxide synthase. Nitric oxide is crucial in regulating the vascular tone, so its shortage leads to endothelial dysfunction, which may then promote atherosclerosis in these individuals [214]. Moreover, the renin-angiotensin-aldosterone axis is upregulated in kidney disease due to the factors outlined in section 2.2.3. RAAS upregulation is known to promote arterial and cardiac stiffness; thus, this could also be one possible link [215]. Another potential connection between DKD and CVD is chronic inflammation, because its role in the pathogenesis of atherosclerosis is well established and it is also induced by many factors in kidney disease, such as subclinical infections, volume overload, oxidative stress, vitamin D deficiency, and sympathetic hyperactivity [216]. Finally, uremic toxins, anemia, and disturbances in the bone and mineral metabolism are additional putative associating, potentially even causal, factors – especially in the context of advanced kidney disease [217].

2.3.4 Mortality

Individuals with type 1 diabetes are also burdened with extensive premature mortality. The life expectancy of young Swedish women and men with type 1 diabetes was recently projected to be 12 and 10 years shorter, respectively, than the life expectancy of their diabetes-free sex- and age-matched counterparts [11]. These findings were analogous to estimations from 2008-2010 from Scotland (-13 years for women, -11 years for men) [218]. However, the longevity of type 1 diabetes has improved substantially during the past decades: Approximations from the US reported a median loss of 27 life-years in the 1970s [219]. Nevertheless, even with a largely acknowledged downward trend in the absolute premature mortality [9,10,202,220,221], not everyone agrees that the relative mortality matched to the general population has fallen over time [202,221].

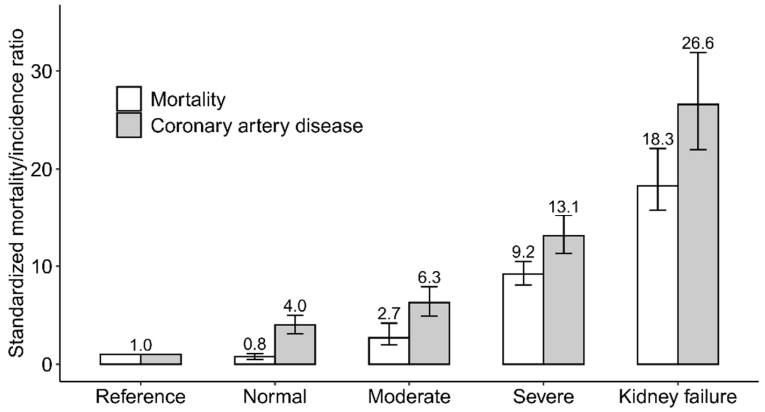


Figure 3 **Standardized mortality ratios and standardized incidence ratios for coronary artery disease at different stages of diabetic kidney disease.** Coronary artery disease was defined as a myocardial infarction or coronary artery procedure. A nondiabetic control population served as the reference group. Normal, moderate, and severe denote stages of albuminuria. The whiskers portray the 95% confidence intervals. Copyright © 2009 American Diabetes Association, from Diabetes 2009;58:1651–8 [12]. Adapted with permission from the copyright holder. The standardized incidence ratios for coronary artery disease stem from Harjutsalo *et al.* [210].

What drives the mortality in type 1 diabetes? Death from acute diabetic complications (principally hypoglycemia and diabetic ketoacidosis) is accentuated during the first decade of diabetes. Nearly 40% of the deaths within ten years since diabetes onset were attributable to acute complications of the disease in a population-based Finnish study covering all individuals with type 1 diabetes diagnosed during 1980-2005 [7]. The corresponding proportion was as high as 74% in Pittsburgh, Pennsylvania, among individuals

with diabetes diagnosed during 1965-79 [8]. The results of these two populations cannot be compared due to many likely confounding factors. However, temporal trends in the mortality associated with acute diabetic complications have been assessed in other cohorts. These reports reveal that, albeit the number of all-cause premature deaths in type 1 diabetes has fallen, the number of fatal acute complications of diabetes shows, unfortunately, no evidence of decline [9,222,223].

In longstanding diabetes, cardiovascular mortality prevails, with ~30 to 65% of the deaths being attributable to CVD [8–10]. Acute myocardial infarction is the most common cause of death within this disease group. However, as for CVD, the perhaps most potent determinant of the mortality risk in longstanding diabetes is the presence and severity of DKD. The average time from first appearance of proteinuria to death, as reported in the early 1980s, was only 7-8 years [14,70], and it was even shorter before that [224,225]. The up-to-date prognosis is better, fortunately, but the detrimental impact of DKD is still apparent. Landmark findings from the FinnDiane Study, presenting data acquired over a median of 7 years, reported that the standardized mortality ratio is already 2.7 at the stage of moderate albuminuria and extends to 18.3 if kidney failure occurs (Figure 3) [12]. Orchard *et al.* from the Pittsburgh EDC Study [13] later replicated the stepwise growing mortality risk with increasing severity of DKD over a follow-up of 20 years. The standardized mortality ratios in the EDC cohort were 6.4 (moderate albuminuria), 12.5 (severe albuminuria), and 29.8 (kidney failure). Of note, in modern times, few individuals with DKD die of kidney-related causes *per se*; CVD, infections, stroke, and malignancies are the most frequent immediate causes of death among individuals who also receive kidney replacement therapy [226,227].

2.4 The metabolism of triglyceride-rich lipoproteins

2.4.1 Overview of lipoproteins and their metabolism

The two most important lipids in plasma, from a clinical point of view, are cholesterols and triglycerides. Cholesterols are important constituents of cellular membranes and serve as precursors for a variety of products, such as steroid hormones, bile acids, and vitamin D. Most of the circulating cholesterols are in esterified form. Triglycerides, in contrast, are esters originating from a glycerol backbone and three free fatty acids. They serve as metabolic fuel for the body and are stored in specialized cells called adipocytes. [228]

The defining attribute of lipids is their insolubility in aqueous media – yet they need to be transported in the blood between tissues of origin or storage and the tissues where they function. This is enabled by the formation of

macromolecular complexes called lipoproteins that are surrounded by a hydrophilic surface composed of a monolayer of phospholipids, free cholesterol, and apolipoproteins. The hydrophobic cholesterols and triglycerides reside in the core. Along with their structural roles in lipoprotein particle formation, the apolipoproteins regulate enzyme activities and act as ligands for membrane receptors. The apoBs are important structural lipoprotein elements, while the other apolipoproteins can be more freely exchanged between lipoprotein particles. [229,230]

The lipoproteins form a continuum of different densities and sizes but are categorized into five main classes based on these features, as Figure 4 illustrates.

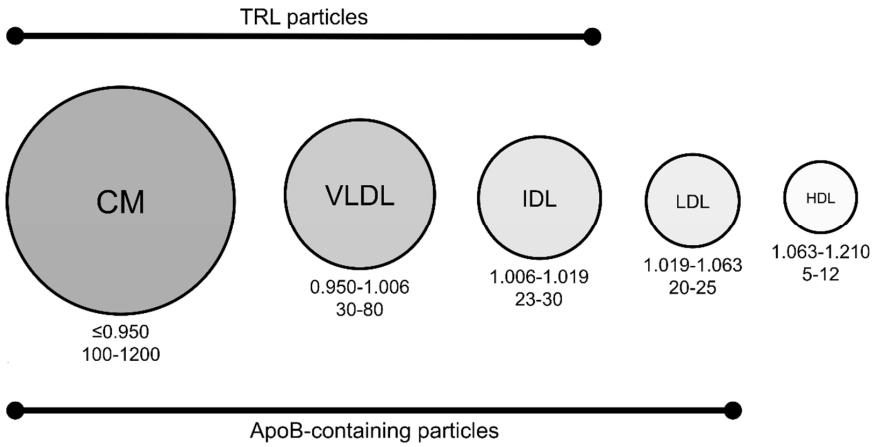


Figure 4 **A pictorial representation of the five main lipoprotein classes.** The numbers indicate densities (upper row; kg/L) and diameters (lower row; nm). TRL, triglyceride-rich lipoprotein; CM, chylomicron; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; ApoB, apolipoprotein B. Densities and diameters: [230].

Chylomicrons are the largest lipoproteins. They are assembled in the enterocytes of the small intestine and transport dietary lipids from the gastrointestinal tract to the peripheral tissues. ApoB-48 is the structural apolipoprotein of chylomicrons (one apoB-48 per chylomicron particle), and the chylomicrons also contain some apoA particles in their nascent, newly established forms. The chylomicrons acquire apoE and apoC-molecules (apoC-I, apoC-II, and apoC-III) from circulating HDL particles during their maturation. The mature chylomicrons proceed through the lymphatic system and enter the bloodstream via the thoracic duct. In adipose, skeletal muscle, cardiac muscle, and lactating mammary tissues, a majority (80-90%) of the triglycerides in chylomicrons are hydrolyzed by the lipoprotein lipase (LPL), an enzyme residing on vascular endothelial cell surfaces. The hydrolysis releases free fatty acids from the triglycerides to be consumed or stored in the

target tissues. The chylomicron particles decrease in size with triglyceride depletion and are consequently termed chylomicron remnants. The triglyceride-depleted chylomicron remnants still contain one molecule of apoB-48 but return most of their apoA- and apoC-content to HDL particles, which in turn enrich the chylomicron remnants with cholesterol esters and apoEs. The chylomicron remnants are eventually taken up by the liver via apoE-mediated endocytosis through the LDL-receptor-related protein (LRP) or the LDL receptor (also called the apoB/E receptor). Of note, apoB-48, in contrast to apoB-100, does not function as a ligand for the LDL receptor. [229,230]

The hepatocytes synthesize and secrete VLDL particles, which contain one molecule of apoB-100 each, together with the excess dietary lipids or dietary carbohydrates that the liver has converted to triglycerides or cholesterol esters. The VLDLs acquire apoCs and apoE from circulating HDLs, and analogously to chylomicrons, the triglycerides of VLDL particles are hydrolyzed by LPL in the vasculature of peripheral tissues. It is noteworthy that the apoB-100 and apoE molecules of mature VLDLs remain in conformations that do not allow binding to the hepatic LDL receptor, while the apolipoprotein conformations of VLDL remnants interact with the receptor. Approximately half of the triglyceride-depleted VLDL remnants are taken up by the liver, whereas the other half (particularly the smaller remnants) continue in the circulation and are now referred to as IDLs. The IDL particles return most of their apoCs to HDL particles in exchange for cholesterol esters. The IDL particles are further catabolized into LDLs with successive triglyceride hydrolysis, this time by the enzyme hepatic lipase. The LDL particles are eventually removed from the circulation via the hepatic LDL receptor or through scavenger receptors in extrahepatic tissues. [229,230]

HDL particles, the smallest and densest of the plasma lipoproteins, are synthesized as lipid-poor, discoid nascent molecules mainly in the liver but also in the intestine. ApoA-I is the core structural protein of HDL and serves as a scaffold for HDL synthesis. The nascent HDL particles acquire free cholesterol during their maturation that effluxes from peripheral cells and is esterified on the HDL particle surface by the plasma enzyme lecithin-cholesterol acyltransferase, also known as LCAT. The subsequent process of cholesterol ester delivery by HDL to the liver for disposal is called reverse cholesterol transport, and because of it, the actions of mature HDL particles are primarily viewed as beneficial. However, as previously described, not all the cholesterol picked up by HDLs is directly transported to the liver: A part is offloaded to atherogenic lipoproteins, particularly in conjunction with the LPL-mediated TRL-processing. This happens either alone or in exchange for triglycerides, and the process is facilitated by the cholesteryl ester transfer protein, or CETP. The TRL remnant particles carry the cholesterol esters to the liver or – particularly if present in excess – they can accumulate in the arterial walls and initiate atherosclerosis. Some of the actions of HDL particles may thus be detrimental. [229–231]

2.4.2 Remnant cholesterol

Definition, measurement, and calculation The TRLs (chylomicrons, VLDL, IDL; Figure 4) are metabolized in the circulation into partially triglyceride-depleted, relatively cholesteryl ester- and apoE-enriched remnant particles, as just described. The major structural apolipoprotein of the remnant particle is apoB-100 when derived from VLDL, whereas apoB-48 serves this purpose in particles that stem from chylomicrons. The following sections will focus on the cholesterol content of TRL particles and particularly their remnants, together referred to as remnant cholesterol.

Some methods for quantification of remnant cholesterol have been developed (nuclear magnetic resonance spectroscopy, electrophoresis, immunoaffinity methods using a monoclonal antibody, and ultracentrifugation); however, not many have been operational for research purposes, and even fewer have thrived in clinical practice [232]. Lipoprotein fractions are derived from continuums of both size and density (Figure 4) where particle sizes partially overlap; thus, true separation of the distinct particles (*e.g.,* of chylomicron remnants from VLDLs) has proven to be very demanding. Additionally, the lipoprotein metabolism is an ongoing, dynamic, and complex interplay, which makes the duplicability of absolute cut-off values difficult. The different direct measurement methods consequently tend to produce very variable estimates of the cholesterol in remnant particles [232–234]. Remnant cholesterol approximation as the non-HDL, non-LDL total cholesterol has been vastly used in clinical study settings [235] and is also the method of choice in this thesis. Calculated remnant cholesterol represents the cholesterol content of VLDL and IDL particles in the fasting state, and chylomicron remnants are included when lipid measurements are performed under non-fasting conditions. The contribution of nascent chylomicrons is minor, because these particles are quickly remodeled to remnants when entering the bloodstream [236].

The advantages of using calculated remnant cholesterol are its low cost and vast availability, because it is derived from a standard lipid profile. The main shortcoming is that calculated remnant cholesterol tends to be higher than the directly measured one. However, Jepsen *et al.*, who studied both calculated and measured remnant cholesterol in a large Danish cohort of individuals with ischemic heart disease [237], reported a strong correlation (r=0.86) between the two methods. The measured form increased by 0.40 (95% confidence interval [CI] 0.39-0.41) mmol/L for every 1 mmol/L increase of calculated remnant cholesterol. Furthermore, Jepsen *et al.* confirmed that both the calculated and the measured remnant cholesterol have a great prognostic value for the all-cause mortality risk and, hence, can equally be used for the purpose [237].

Remnant cholesterol vs. triglyceride-rich lipoprotein cholesterol Some experts argue that the term remnant cholesterol is over-simplified, and the term

triglyceride-rich lipoprotein cholesterol should be used instead. Accordingly, the term *remnant* should only be used when referring to already partially delipidated TRL particles, or with even stricter definitions, solely for the triglyceride-depleted particles that are no longer following the regular lipoprotein removal route. [233]

However, there is a critical counterargument: When newly produced chylomicrons and VLDL particles enter the bloodstream, LPL immediately starts the degradation of triglycerides in the particles. Thus, virtually all TRLs are remnants to some degree – except in the extremely rare case of complete LPL deficiency. With this in mind, the term *remnant cholesterol* has a physiological ground and, perhaps even more importantly, is likely easier to grasp by the patients and clinicians than the term *triglyceride-rich lipoprotein* cholesterol. [233]

The term *remnant cholesterol* is consequently used throughout this thesis when referring to the non-HDL, non-LDL cholesterol.

Remnant cholesterol and atherosclerosis The indisputable involvement of LDL-cholesterol in CAD was discovered in the 1950s and was long accepted as the unrivaled root of atherosclerosis [238]. It was not until two decades later that Professor Zilversmit introduced his hypothesis that atherosclerosis is a multifactorial process evolving not only as a result of LDL particle accumulation but also as a postprandial phenomenon due to the particularly atherogenic cholesterol-loaded chylomicrons and their remnants [239]. Professor Zilversmit also cautiously implied in his seminal research that VLDL particles may also be atherogenic.

The focus of atherosclerosis research shifted after the launch of the so-called *postprandial hypothesis* towards high triglyceride [236] and/or low HDL-cholesterol concentrations [240]. However, clinical trials targeting HDL-cholesterol failed to reduce the cardiovascular burden [241,242], and Mendelian randomization studies have been unsupportive of a causal relationship between HDL-cholesterol and CVD outcomes [243,244]. It is also unlikely that triglycerides are a direct cause of atherosclerosis, the rationale of which is as follows: Retention of apoB-100 and apoB-48-containing lipoproteins has been encountered in atherosclerotic plaque composition analyses in both animal models [245] and human tissue studies (femoral and carotid endarterectomy samples [246] and postmortem aortic samples [247]). Of note, the entrapped apoB-100 has been detected in particles of both LDL and VLDL dimensions [247]. However, as nascent chylomicrons and large VLDL particles are restricted by their large sizes (>70 nm) to enter the arterial intima [194,248,249], the current interpretation is that it is their remodeled, *triglyceride-depleted* remnants that penetrate the endothelial barrier and accumulate in atheromas. With the emerging of remnant cholesterol as a plausible culprit, hypertriglyceridemia and low HDL-cholesterol are, to date, primarily viewed as biomarkers in the context of atherosclerosis.

Several other characteristics of the remnant particles also favor their proatherogenic role. For instance, when entering the subendothelial space, the remnant particles are susceptible to entrapment within the connective tissue matrix through apoE/apoB-proteoglycan binding [250]. The remnant particles, in contrast to LDL particles, can then be engulfed by macrophages without prior oxidative modification [22]. However, not all the atherogenic properties of these lipoprotein particles are attributable to the cholesterol content: The hydrolysis of their triglycerides releases tissue-toxic free fatty acids and monoacylglycerols causing local inflammation at the arterial wall and, via this, enhances the development of vulnerable atherosclerotic plaques [22,251].

Remnant cholesterol concentrations have been strongly predictive of various vascular disease endpoints in large-scale epidemiological study settings, including ischemic heart disease [252], ischemic stroke [253], and chronic kidney disease [254]. High remnant cholesterol concentrations are also associated with the total atherosclerotic burden as assessed by computer tomography coronary angiography – even in individuals with LDL-cholesterol at an optimal stage [255]. Importantly, results from a Mendelian randomization study by Varbo *et al.* have proposed that the relationship between calculated remnant cholesterol and ischemic heart disease is causal [252]. The impact of remnant cholesterol is further supported by findings in individuals with familial dysbetalipoproteinemia, a genetic disorder leading to dysfunctional apoE and characterized by elevated remnant lipoproteins and peripheral leukocytosis, increased intracellular lipid-accumulation in plasma monocytes, enhanced monocyte activation, and arterial wall inflammation – all key components in the atherosclerotic plaque formation [256]. In fact, patients with familial dysbetalipoproteinemia have up to 10-fold odds of developing atherosclerotic heart disease, compared to the general population [257].

Remnant cholesterol, type 1 diabetes, and diabetic complications Given the renewed interest in remnant cholesterol as a potential cause of atherosclerosis and the many studies published in the area during recent years, type 1 diabetes has been surprisingly poorly represented. Despite impaired postprandial metabolism of apoB-48 having been observed in type 1 diabetes [258,259], the knowledge of remnant cholesterol *per se* is limited. Remnant cholesterol in the development of diabetic complications, especially the microvascular ones, has been particularly sparsely described in this patient population. A cross-sectional analysis of the DCCT/EDIC trial revealed that the study participants with type 1 diabetes and concomitant DKD were characterized by high VLDL particle concentrations (particularly the smaller particle subclasses) and IDL particle concentrations (in men but not in women) [145], but other than that, the topic has so far been overlooked. Furthermore, no studies have specifically assessed the association between remnant cholesterol concentration and macrovascular outcomes in type 1 diabetes; however, in this case, findings can

likely be extrapolated from the general population or from the studies that have involved subjects with type 2 diabetes [260,261].

Remnant cholesterol as a therapeutic target Three lipid-lowering medication classes have appeared efficient in lowering remnant cholesterol concentrations, either alone or as combination therapy. First, a *post-hoc* analysis of the TNT (*Treating to New Targets*) Trial showed that atorvastatin therapy reduces remnant cholesterol concentrations by ~10 to 25% in a dose-dependent manner [262]. Second, recent findings suggest that combining simvastatin with ezetimibe confers greater benefits than statin only [263]. Third, PCSK 9 inhibitors appear particularly potent: In a study on patients receiving treatment with PCSK 9 inhibitors for atherosclerotic CVD and/or familial hypercholesterolemia, 24 to 32% lower remnant cholesterol (depending on the determination method) was observed after at least three doses of the medicine [264].

The findings from the TNT Trial encouragingly hinted that the drop in remnant cholesterol translated into CVD risk reduction independent of changes in LDL-cholesterol [262]. However, the fact that LDL-cholesterol levels are also affected by the lipid-lowering agents previously listed makes it practically impossible to determine how much of the CVD risk reduction is mediated through remnant cholesterol alone. Attacking apoC-III may bring some clarity to this question, because genetic apoC-III inhibition appears to confer lipid alterations more specific to remnant cholesterol than what has been established before. This topic will be reviewed next.

2.4.3 Apolipoprotein C-III

Molecular properties ApoC-III is a small 8.8 kDa, 79-amino acid protein [265]. It circulates as a component of apoB-containing lipoproteins and is dynamically redistributed between these and HDL particles, especially in conjunction with the triglyceride hydrolysis of TRLs, as just described. The majority of apoC-III resides on HDL particles in normolipidemic individuals, whereas in hypertriglyceridemic individuals, most is found on TRLs [265]. The normal range of apoC-III concentration has not been officially defined, but a study by Nicolay *et al.* outlined the reference interval of total serum apoC-III as 9.25 ± 3.32 mg/dL, derived from healthy, normolipidemic controls [266].

Synthesis and its regulation ApoC-III is encoded by the *APOC3* gene on chromosome 11q23. The gene is expressed in the liver and to a lesser extent in the intestine. Several factors are known to modulate the transcription of the *APOC3* gene; one such is glucose. Circulating glucose induces the gene transcription by a mechanism comprising the transcription factors carbohydrate response element-binding protein and hepatocyte nuclear factor-4α [267]. Interestingly, insulin has an opposite, repressing effect, and it

appears that this negative regulation is mediated through phosphorylation of the nuclear transcription factor Forkhead box O1. Polyunsaturated fatty acids also downregulate *APOC3* expression through the same pathway, whereas saturated fatty acids increase the hepatic apoC-III production in mice and men [268].

Role in lipoprotein metabolism ApoC-III acts through several pathways to impair the clearance and subsequently increase the plasma resident time of TRLs and their remnants. Findings from kinetic studies have revealed that the apoC-III concentration is, in fact, the strongest predictor of the TRL catabolism rate in the body [269]. ApoC-III thus plays a crucial role in the triglyceride homeostasis. Figure 5 shows a pictorial representation of the actions of apoC-III, which will be reviewed below.

First, apoC-III halts the hepatic TRL clearance via LDL family receptors (the LDL receptor and LRP1) through displacement of the apoE molecule from the lipoprotein particle or by direct interference with the apoE-receptor binding [270]. This is the most established role of apoC-III in the lipoprotein metabolism. It has also been suggested that apoC-III masks the surface-embedded apoB-100 molecule, which is another potential ligand for the LDL receptor on hepatocytes [271]. ApoC-III further impairs the TRL clearance by LPL inhibition. Several proposed underlying mechanisms for this exist [268,272], such as that apoC-III competes with apoC-II (activator protein for LPL) and/or directly with LPL for the binding of TRLs. Moreover, at high concentrations, apoC-III may also inhibit hepatic lipase [273].

It remains unclear whether apoC-III affects the TRL synthesis or not. Some studies from animal models and cultured cells have suggested that apoC-III enhances the hepatic secretion of VLDL [274]; however, suppression of *APOC3* expression in mice with an antisense nucleotide agent did not influence VLDL secretion in one study [275]. Studies on this topic in humans are lacking. Therefore, the impaired TRL clearance is regarded as the primary route through which the effects of apoC-III are mediated.

ApoC-III and atherosclerosis Compelling evidence from studies has associated apoC-III with CVD manifestations, including incident [276,277] and recurrent myocardial infarctions [278], stroke [279], and cardiovascular death [280]. Statin therapy reduces apoC-III concentrations by ~20% [265,281], although the associations between apoC-III and CVD events have appeared independent of lipid-lowering treatment. ApoC-III has, therefore, been suggested as a marker of at least part of the residual CVD risk in statin-treated individuals [282].

Genetic studies have provided additional support for the apoC-III-CVD-hypothesis. Two large studies identified three loss-of-function mutations in the *APOC3* gene and subsequentially revealed ~40% lower concentrations of fasting and nonfasting triglycerides in heterozygote carriers of the variants [283,284]. Importantly, the CVD risk in these individuals was reduced to the

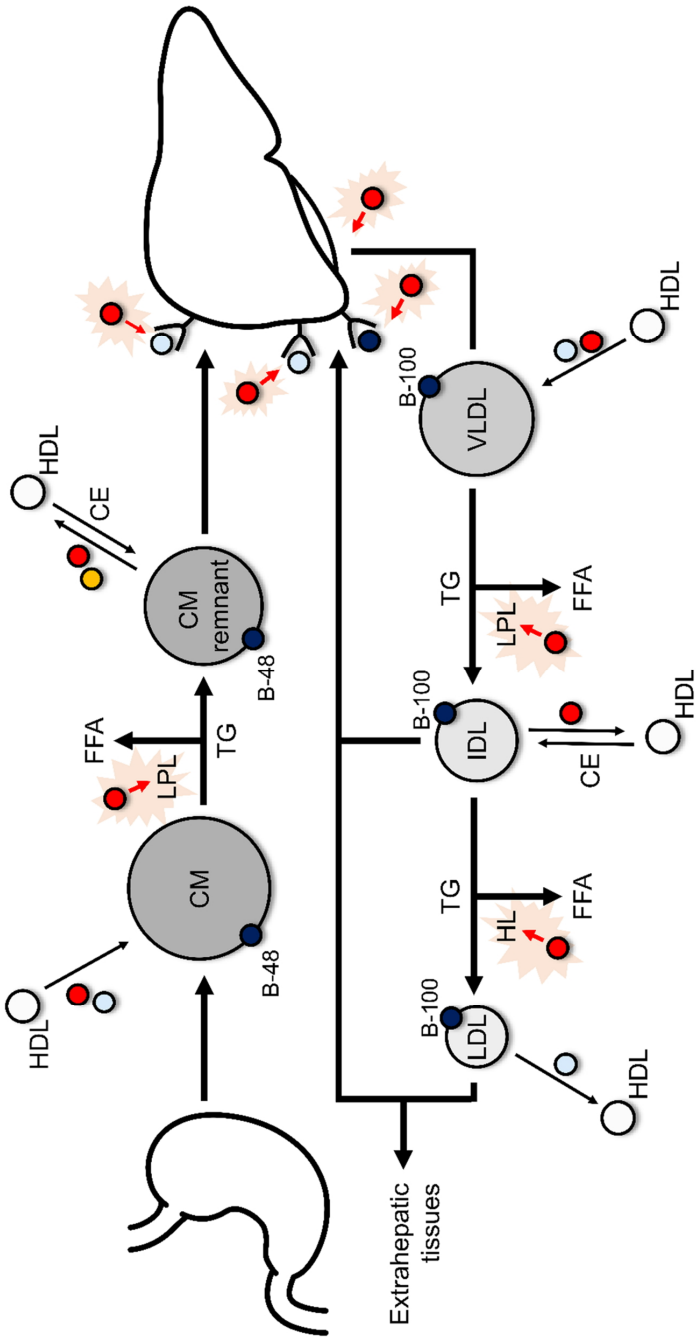


Figure 5 **An overview of the roles of apolipoprotein C-III (red arrows) on the lipoprotein metabolism.** CE, cholesteryl ester; CM, chylomicron; FFA, free fatty acid; HL, hepatic lipase; TG, triglyceride. The red spheres denote apoCs, light blue spheres apoEs, dark blue spheres apoBs, and yellow spheres apoAs. The gastrointestinal tract and the liver are pictorially represented.

same magnitude. Remnant cholesterol concentrations were not directly evaluated in the studies, but due to the reasons outlined earlier, the authors interpreted the triglyceride concentrations as a proxy for the cholesterol in remnant lipoprotein particles. It is noteworthy that in loss-of-function mutation carriers of the *APOC3* gene, effects on LDL-cholesterol concentrations have been more modest (-4% in a meta-analysis by Wulff *et al.* [285]), which has led to an interpretation that the cut in CVD risk is primarily conferred by the change in remnant cholesterol.

It is assumed that apoC-III predominantly contributes to atherogenesis by its detrimental effects on the lipoprotein metabolism. Besides that, apoC-III bound to apoB-containing particles is thought to increase the affinity between these and the arterial wall proteoglycans, thus boosting the vascular LDL/TRL particle retention [194,286]. Furthermore, apoC-III has been associated with several inflammatory markers and, through increasing the endothelial cell expression of vascular adhesion molecules, is believed to promote the connection between monocytes and endothelial cells [287]. All these actions are proatherogenic and could explain the contribution of apoC-III to CVD.

ApoC-III, type 1 diabetes, and diabetic complications Considering the impact of glucose on the *APOC3* gene expression as well as the effects of apoC-III on the lipoprotein metabolism, apoC-III has emerged as a putative link connecting hyperglycemia, hypertriglyceridemia, insulin resistance, and CVD in type 2 diabetes. However, individuals with type 1 diabetes have also raised apoC-III concentrations [288,289], and apoC-III has also been linked to an elevated CVD risk in this patient population. Kanter *et al.* lately published an interesting study from the Coronary Artery Calcification in Type 1 Diabetes cohort that proposed apoC-III as a stronger predictor of CVD events than what plasma triglyceride concentrations are [23]. Using diabetic mouse models, the researchers showed that insulin deficiency translates to nearly two-fold apoC-III levels and, importantly, that the reduction of apoC-III impedes atherogenesis [23]. An even more recent study from the same cohort demonstrated that serum apoC-III is positively associated with insulin resistance and coronary artery calcium [290] – hence, further reinforcing the link between apoC-III and atherosclerosis in type 1 diabetes.

Less is known about the relationship between apoC-III and microvascular complications in type 1 diabetes. ApoC-III concentrations are known to be particularly high among those patients who have advanced kidney failure, which is in line with the multiple other lipid and lipoprotein abnormalities of these individuals [291]. Data from the DCCT/EDIC cohort have shown a correlation between apoC-III and microvascular diabetic complications, depicted by AER and the severity of diabetic retinopathy, with a cross-sectional study design [292]. However, besides this the knowledge is sparse, and whether the apoC-III concentration predicts the progression of DKD has not been evaluated. Furthermore, it is unknown whether the presence of DKD modulates the association between apoC-III and macrovascular endpoints.

ApoC-III as a therapeutic target As mentioned earlier, statins reduce apoC-III concentrations to some extent, although the effect is limited. The same applies to fibrates and ezetimibe [265,281]. More promising results have been obtained with *Volanesorsen*, an antisense nucleotide agent designed to knock down the production of apoC-III by binding selectively to its messenger RNA. The agent is approved in Europe for the treatment of familial chylomicronemia syndrome, a genetic disorder characterized by LPL deficiency, in which the agent efficiently reduces plasma triglycerides (up to 80%) and subsequently lowers the pancreatitis risk [293]. Several clinical trials are currently investigating the effects of Volanesorsen therapy on, for instance, partial lipodystrophy and type 2 diabetes with promising initial results, and the agent has been highlighted as a promising future CVD risk modulating drug. No investigations encompassing individuals with type 1 diabetes have been initiated yet.

3 AIMS OF THE STUDY

The objective of this thesis was to seek a holistic view of the contemporary natural history – the incidence, the progression, and the regression – of DKD in type 1 diabetes. The progression of kidney disease was evaluated in light of the TRL metabolism. Furthermore, the relationship between the TRL metabolism, DKD, cardiovascular events, and mortality was explored.

The specific aims were as follows:

I To assess the diabetes duration-specific incidence rate patterns, cumulative incidences, and temporal fluctuations of these for moderate and severe albuminuria in type 1 diabetes.

II To study the associations between remnant cholesterol concentration, remnant cholesterol variability, apoC-III, and the progression of DKD in type 1 diabetes.

III To investigate the relationship between remnant cholesterol, apoC-III, and cardiovascular events, as well as all-cause mortality in type 1 diabetes. We specifically aimed to evaluate the impact of DKD on the associations.

IV To elucidate the rate of regression of albuminuria in type 1 diabetes, as well as the association between albuminuria regression and the occurrence of cardiovascular events and mortality.

4 SUBJECTS AND STUDY DESIGNS

4.1 The population-based cohort (Study I)

4.1.1 Study cohort

The population-based cohort in Study I comprises a stratified random sample of 1,500 individuals among all individuals diagnosed with type 1 diabetes before the age of 15 years during 1970-99 in Finland. The calendar-year diagnosis cohorts 1970-79, 1980-89, and 1990-99 are represented in equal extents (500 individuals from each diagnosis cohort), as are men and women (250 and 250 individuals from each diagnosis cohort). The sex distribution follows that of the sampling frame closely: The proportion of men in the sampling frame population is 53.9%.

The sampling frame is compiled from successive register-based national administrative and health care registers [29,30]. Its primary sources are the Finnish Institute for Health and Welfare's Care Register for Health Care (named the Hospital Discharge Register 1969-1993) and the Social Insurance Institution's entitlements to elevated reimbursements for antidiabetic medications. Of note, insulin treatment has been fully reimbursed for individuals with type 1 diabetes in Finland since 1964, which makes the case ascertainment in the sampling frame virtually complete. Unique personal identifiers that are assigned to every resident of Finland have been used to link data between the registries. Classification of the diabetes type within the sampling database is primarily accomplished using diagnosis codes: ICD-9 code 250B, ICD-10 code E.10/O24.0, and/or ICPC-2 code T89 denote type 1 diabetes.

The sample size was based on power calculation and represents 14.4% of the sampling frame population (n=10,439). The sampling was conducted with the SURVEYSELECT procedure in SAS.

4.1.2 Study design

All Finnish residents are entitled to medical care services in the public health care system, including screening and diagnostic tests, treatment, and care equipment prescribed in treatment plans. Following international recommendations, the Finnish Care Guidelines also advise screening for the presence of albuminuria at least annually after a diabetes duration of five years among individuals with type 1 diabetes [294].

Medical records from diabetes clinic visits were systematically reviewed for the ascertainment of DKD stage in Study I. The medical records were

requested on four occasions between May 2017 and December 2020. Data from the diabetes onset onwards were collected until the last available diabetes check-up; that is, until 2017-2020, death, or a time point earlier than this if the subject was lost to follow-up due to emigration, discontinuation of albuminuria screenings, or some other cause. The diabetes clinics that were contacted for medical records were either part of public health care centers or regional-, central-, or university hospitals. These were identified through the Care Register for Health Care or based on the municipalities of residence, as retrieved via the Population Register. Ultimately, medical records were acquired from all five university hospitals, all 16 central hospitals, 39 regional hospitals, and over 100 health care centers all over Finland.

4.1.3 Inclusion and exclusion criteria

The participant selection process is visually outlined in the first panel of Figure 6. Five individuals were excluded from the original sample due to characteristics of the clinical course that did not correspond to type 1 diabetes (*e.g.,* ceased need of insulin therapy during follow-up). Three individuals developed a kidney disease other than DKD and were therefore excluded. Twenty individuals had partaken in too few albuminuria screenings (less than three) for conclusions to be drawn. Moreover, no medical records were available for 42 study subjects; of these, four had emigrated before the initiation of annual albuminuria screenings; the medical records of 31 had been discarded; and the medical records for the remaining seven individuals were unavailable for other or unknown causes. Accordingly, 462 (92.4%), 481 (96.2%), and 487 (97.4%) individuals were included from the calendar-year cohorts 1970-79, 1980-89, and 1990-99, respectively, thus representing 95.3% of the original study sample.

Prospective data on severe albuminuria were available for all 1,430 included subjects. However, moderate albuminuria was assessed only in those diagnosed with diabetes in 1980 or later due to the limited accessibility of early albuminuria determinations among the individuals with onset of diabetes in 1970-79. Information on moderate albuminuria was available in altogether 961 individuals.

The attained follow-up time for moderate albuminuria was 20,963 person-years (median [interquartile range; IQR] 21.8 [16.8-27.6] years) and 37,869 person-years (median 25.1 [19.9-32.1] years) for severe albuminuria.

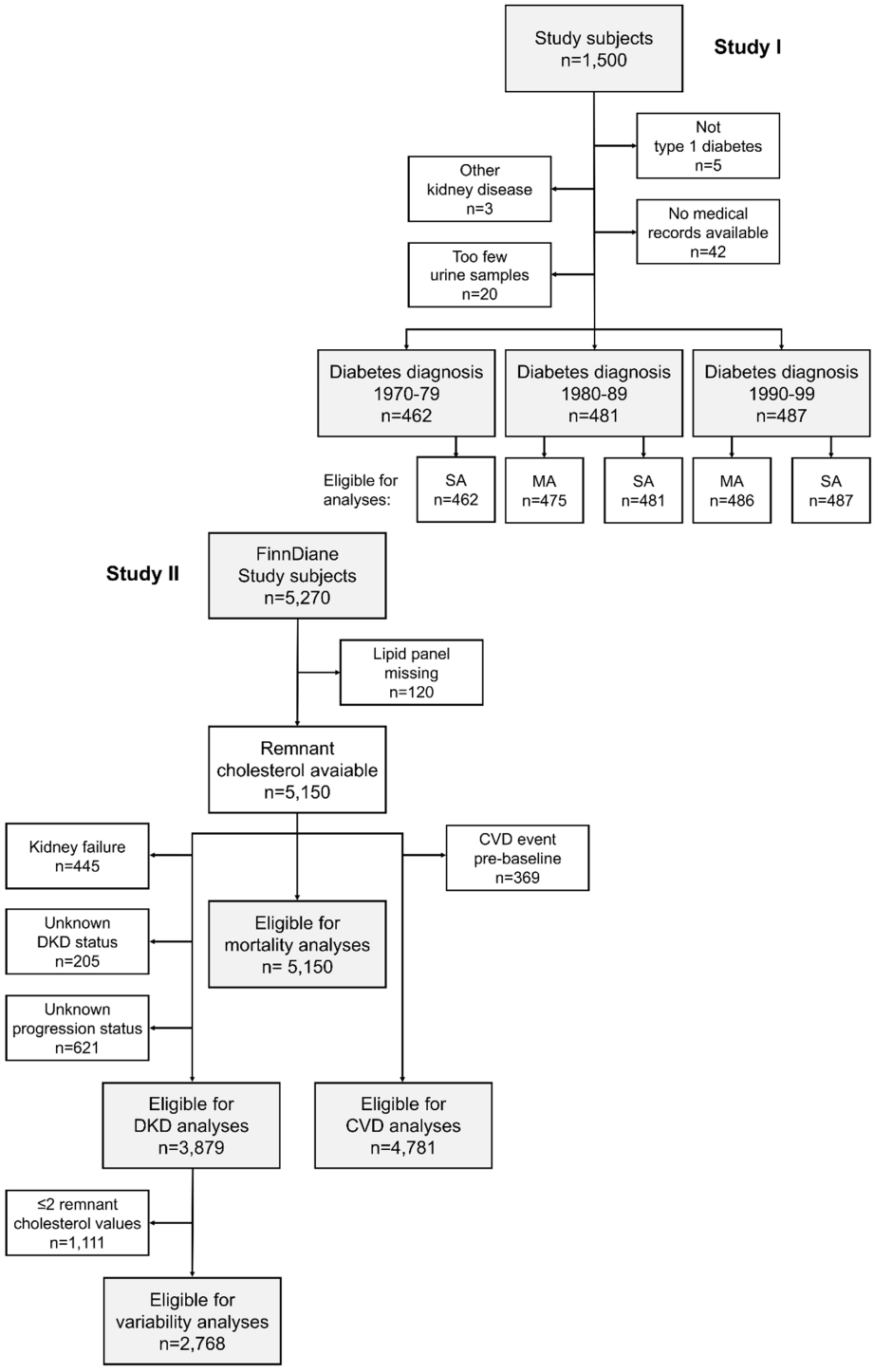
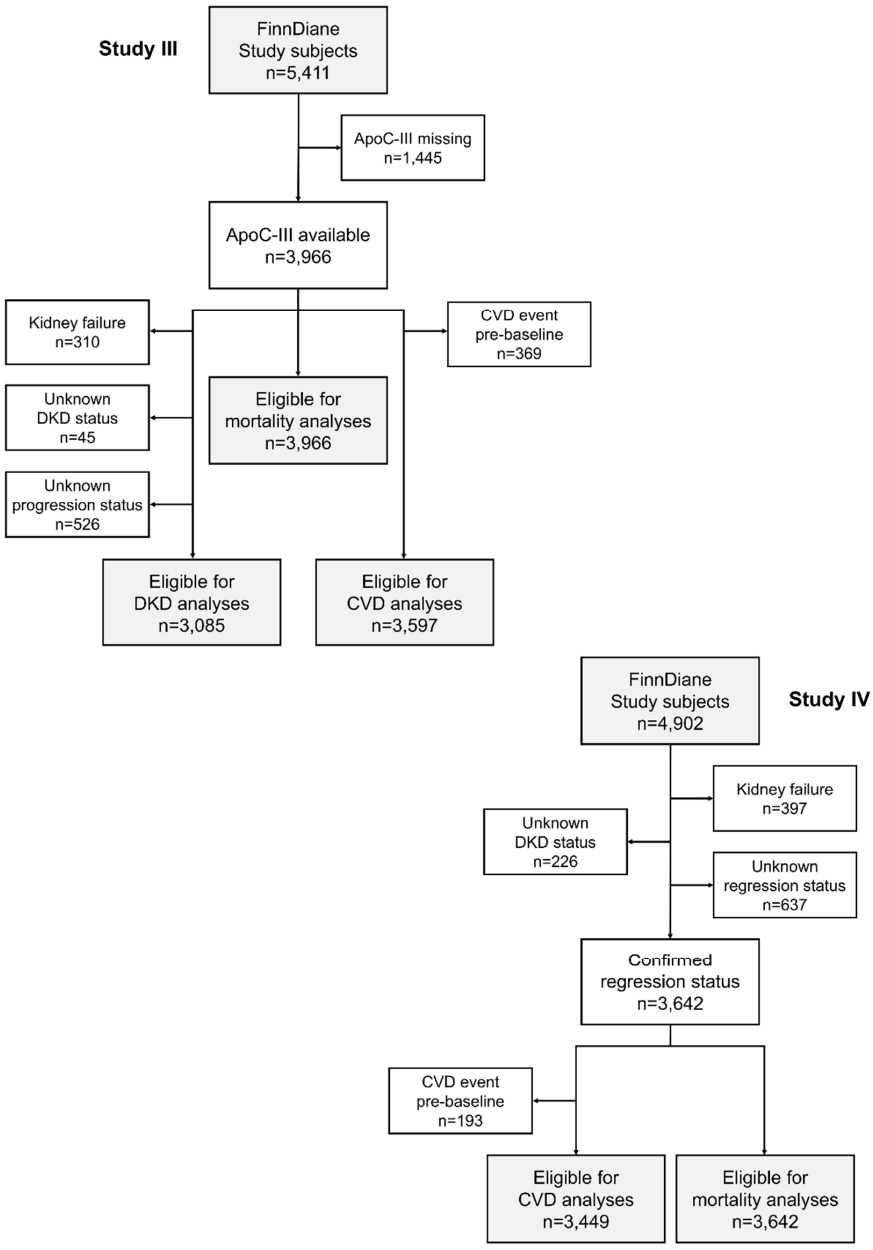

Study subjects
n=1,500
Study I
Not
type 1 diabetes
n=5
Other
kidney disease
n=3
No medical
records available
n=42
Too few
urine samples
n=20
Diabetes diagnosis
1970-79
n=462
Diabetes diagnosis
1980-89
n=481
Diabetes diagnosis
1990-99
n=487
Eligible for
analyses:
SA
n=462
MA
n=475
SA
n=481
MA
n=486
SA
n=487
Study II
FinnDiane
Study subjects
n=5,270
Lipid panel
missing
n=120
Remnant
cholesterol avaiable
n=5,150
Kidney failure
n=445
CVD event
pre-baseline
n=369
Unknown
DKD status
n=205
Eligible for
mortality analyses
n= 5,150
Unknown
progression status
n=621
Eligible for
DKD analyses
n=3,879
Eligible for
CVD analyses
n=4,781
≤2 remnant
cholesterol values
n=1,111
Eligible for
variability analyses
n=2,768

Figure 6 **Compilation of the included cohorts in Studies I-IV** (*continuation from the previous page*). SA, severe albuminuria; MA, moderate albuminuria



4.2 The FinnDiane Study cohort (Studies II-IV)

4.2.1 Study cohort

The Finnish Diabetic Nephropathy (FinnDiane) Study is a nationwide, multicenter, longitudinal study aiming to answer the question: *Why do one-third of individuals with type 1 diabetes develop diabetic nephropathy*? More precisely, the study was launched in November 1997 to unravel the pathophysiological mechanisms underlying complications of type 1 diabetes and to identify their clinical, environmental, metabolic, and genetic risk factors. The emphasis is on kidney disease, although comprehensive research on diabetic retinopathy, neuropathy, and macrovascular complications is also carried out.

The enrollment of participants in the FinnDiane Study is ongoing. Individuals with type 1 diabetes have been invited to participate in connection with their regular visits to general, diabetes, and renal outpatient clinics. The FinnDiane Study covered 5,517 study participants from 78 centers by May 2022, including numerous primary health care center units and hospitals from the secondary and tertiary care level in the public sector, as listed in the Appendix. The number of study participants corresponds to roughly 10% of all individuals with type 1 diabetes in Finland. Although the distribution of study participants closely follows that of the general population in Finland, the FinnDiane Study cohort is not population-based by strict definitions.

4.2.2 Study design

Figure 7 outlines the FinnDiane Study protocol.

The cornerstone of the FinnDiane Study is its study visit occurring at the central research laboratory in Helsinki or a local study center elsewhere in Finland. A trained study nurse or attending physician gathers information at the study visit relating to the study participant's treatment and follow-up of diabetes, the history of diabetic complications, other medical backgrounds, current and previous medication, lifestyle habits (such as smoking and alcohol use), and family history with respect to vascular diseases. A thorough clinical examination is performed. Blood is also drawn after a light breakfast. The participant is asked to bring a 24-hour urine sample for the determination of AER, among other urinary clinical variables.

Eligible FinnDiane study participants have been re-examined regularly since 2004 for the longitudinal part of the study. By May 2022, 36% of the study participants had attended two regular study visits, 11% three visits, and 1.9% four study visits. The median between-visit time was 6.7 years (range 0.5-22.2 years). In addition to the revisits, medical records for all FinnDiane participants are retrieved from the hospitals or health care centers where the

individuals attend their diabetes-related check-ups and are reviewed by FinnDiane personnel to obtain information about albuminuria progression status as well as serial lipids, serum creatinine, and HbA_{1c} measurements. The cardiovascular events, retinal laser photocoagulations, transitions to kidney replacement therapy, and mortality data are retrieved from national registers and linked using unique personal identity codes.

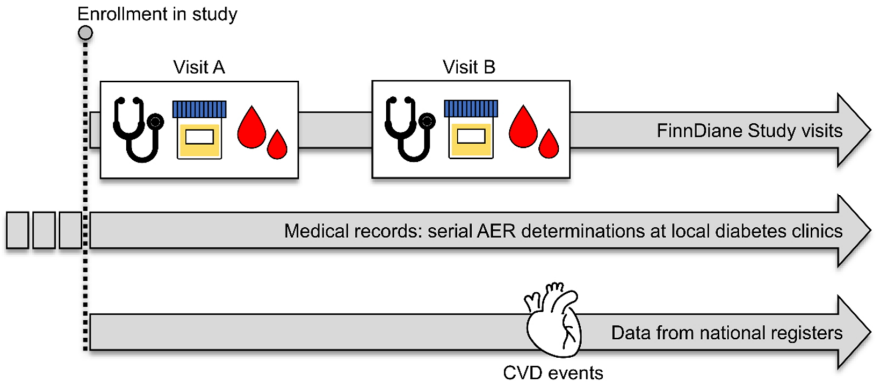


Figure 7 **Schematic diagram of the FinnDiane Study protocol**. After entering the study (dashed line), the participant partakes in a study visit ("Visit A") at a FinnDiane center, where he or she undergoes a thorough medical examination. Blood is drawn and urine is collected at the visit. The attending nurse or physician gathers the significant medical history through validated questionnaires and from available medical records, from which the most advanced DKD stage is set. The subsequent development of DKD (progression/no progression) is followed through medical records and data retrieved from potential revisits (a second visit, "Visit B," takes place for this hypothetical study participant). Additionally, the occurrence of cardiovascular disease (CVD) outcomes, along with kidney failure outcomes, retinopathy outcomes, and vital status, are regularly checked from national registers.

4.2.3 Inclusion and exclusion criteria

The participant selection processes for Studies II-IV appear in Figure 6.

It is noteworthy that the FinnDiane Study subjects have not been preselected based on the presence or absence of DKD or other diabetic complications; the only requirement for participation is a diagnosis of type 1 diabetes. However, only those with diabetes onset under the age of 40 and who transitioned to permanent insulin treatment within one year of diagnosis were included in Studies II-IV to ensure that the diabetes classification had been set correctly.

By January 2020, 5,270 individuals had participated in the FinnDiane Study and formed the original cohort for Study II. A total of 120 individuals had an incomplete baseline lipid profile and were therefore excluded.

The first FinnDiane visit was chosen as the baseline in studies II and IV. However, apoC-III measurements were introduced to the FinnDiane laboratory panel at a later stage, so the first visit with an apoC-III measurement was chosen as the baseline in Study III. The initial cohort for Study III comprised all the 5,411 individuals with at least one FinnDiane study visit by December 2020. An apoC-III measurement was available for 3,966 participants. The first FinnDiane visit (visit A) served as the study baseline in the case of 3,226 participants, whereas for 728 and 12 individuals, the baseline was set at the second (visit B) and third visit (visit C), respectively. The CVD analyses in Study IV included only the 3,597 without a history of CVD events prior to the study visit that was set as the baseline.

The initial cohort for Study IV comprised all individuals with confirmed type 1 diabetes who had participated in the FinnDiane Study prior to July 2015. The ones with kidney failure or unknown DKD status at baseline were excluded. An albuminuria regression status (yes/no) was set for the 913 individuals with a history of moderate or severe albuminuria (438 and 475, respectively), and 2,729 study participants with normal AER at baseline served as the reference group in the CVD and mortality analyses. Thus, the final study cohort consisted of 3,642 individuals. The individuals who had experienced a CVD event prebaseline were excluded from the CVD analyses.

A total of 3,189 individuals were included in all three FinnDiane studies of this doctoral thesis (Figure 8).

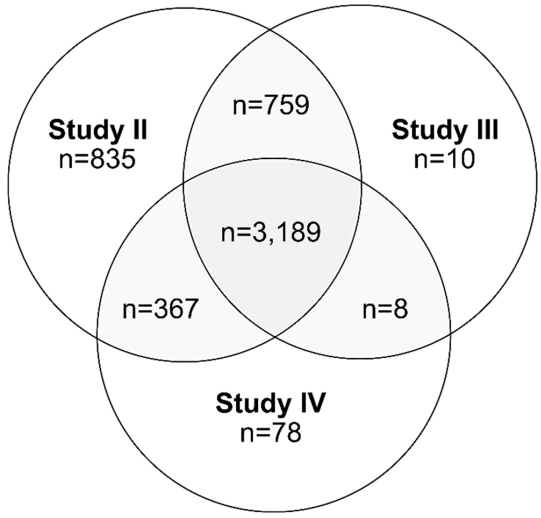


Figure 8 **Venn diagram portraying the relationship between the Study cohorts II-IV** (*i.e.*, the FinnDiane studies of this thesis).

4.3 Ethical aspects

Study I was approved by the Finnish Institute for Health and Welfare (THL/786/6.02.00/2016) and Statistics Finland (TK53-26-16). Informed consents were not required due to the register-based study setting. The research plan of the FinnDiane Study (Studies II-IV) was approved by the Ethical Committee of the Helsinki and Uusimaa Health District and the local ethics committees of all participating centers (Appendix). The study was performed according to the Declaration of Helsinki. All participants provided their written informed consent before participation.

5 METHODS

5.1 Diabetic kidney disease

5.1.1 Albuminuria

The AER stage was categorized into normal range, moderate albuminuria, or severe albuminuria according to the international reference limits that appear in Table 2. Two out of three abnormal determinations were required for a diagnosis of moderate or severe albuminuria, and the time of onset was set as the second positive measurement in a series of three diagnostic samples. Urine samples collected during urinary tract infections, acute febrile illnesses, menstruation, pregnancy, severe hyperglycemia, or within 24 hours of strenuous physical exercise were excluded.

In the absence of albuminuria determinations, a 24-hour urinary protein excretion rate >500 mg and/or a positive reading using a reagent strip testing for urinary protein was considered indicative of severe albuminuria. Proportions of the different albuminuria determination methods by calendar-year diagnosis cohort in Study I appear in Table 3.

Table 2 Reference limits for categorization of albuminuria [59,295].

	24-h collection (mg/24h)	Timed overnight collection (μg/min)	Spot collection (mg/mmol creatinine)
Normal AER	<30	<20	<3
Moderate albuminuria (microalbuminuria)	30-299	20-199	3-29
Severe albuminuria (macroalbuminuria)	≥300	≥200	≥30

The AER was determined by radioimmunoassay (Pharmacia, Uppsala, Sweden) at the FinnDiane visits until 2002, and thereafter by an immunoturbidimetry method (Hitachi 911 analyzer, Roche Diagnostics, Hoffman-La Roche, Basel Switzerland).

5.1.2 Kidney failure

Kidney failure was defined as the initiation of maintenance dialysis treatment or kidney transplantation. The information on kidney replacement therapy was retrieved from the National Care Register of Health Care.

Table 3 Proportions of the different albuminuria determination methods in the three diagnostic urine samples in Study I.

	24-h collection (mg/24h)	Timed overnight collection (µg/min)	Spot collection (mg/mmol creatinine)	Proteinuria determination*
Severe albuminuria				
Total cohort	*10.9%*	*54.1%*	*16.3%*	*18.8%*
1970-79	16.0%	49.7%	5.1%	29.1%
1980-89	7.0%	59.4%	23.4%	10.2%
1990-99	4.6%	55.4%	32.3%	7.7%
Moderate albuminuria				
Total cohort	*10.0%*	*70.9%*	*19.1%*	*NA*
1980-89	13.4%	73.2%	13.4%	NA
1990-99	6.5%	68.6%	24.9%	NA

*24-hour urinary protein excretion rate >500 mg and/or a positive reading using a reagent strip testing for urinary protein was diagnostic for severe albuminuria. NA, not applicable. Adapted from Study I (Supplementary appendix 3 Table 1) with permission from the copyright holder.

5.1.3 Progression of diabetic kidney disease

Studies I, II, and III evaluated progression of DKD. Progression was defined as a change to a more advanced stage of albuminuria or as the initiation of kidney replacement therapy. DKD progression is represented by red arrows in Figure 9.

5.1.4 Regression of albuminuria

Study IV assessed regression of albuminuria. At the first FinnDiane visit (*i.e.*, the study baseline), a 24-hour urine sample was available, along with the history of DKD via medical records and questionnaires. The medical records included both 24-hour urine collections, timed overnight collections, and spot samples. The most advanced prebaseline stage of albuminuria was set for each participant based on two out of three consecutive urine samples, with careful evaluation of the potential confounding circumstances presented previously. A revised albuminuria classification was also performed, based only on the three most recent urine collections at baseline (including the 24-hour collection at the first FinnDiane study visit). Regression of albuminuria was defined as a change from a prior higher to a lower classification of albuminuria at baseline, that is, from moderate albuminuria to normal AER, severe to moderate albuminuria, or from severe albuminuria to normal AER (blue

arrows in Figure 9). Figure 10 illustrates an example of regression of albuminuria.

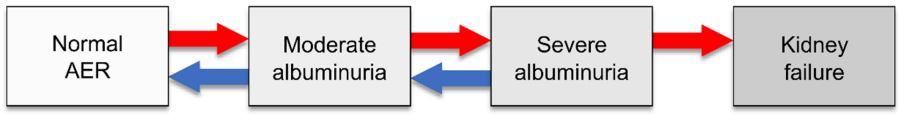


Figure 9 **The natural history of diabetic kidney disease.** Red arrows denote progression, blue arrows regression. AER, albumin excretion rate.

5.1.5 Estimated glomerular filtration rate

Serum creatinine was determined using a kinetic Jaffé reaction (Hitachi 911 analyzer, Boehringer Mannheim, Mannheim, Germany) until January 2002; thereafter, a photometric, enzymatic method (Hitachi 917 or Modular analyzer, Boehringer Mannheim/Roche Diagnostics, Basel, Switzerland) was used. The correlation coefficient between the methods was 0.988, and the formula $S\text{-}creatinine_{CORRECTED} = (0.953 \times S\text{-}creatinine_{JAFFÉ}) - 7.261$ was used to harmonize the measurements. The GFR was estimated using the CKD-EPI equation [65].

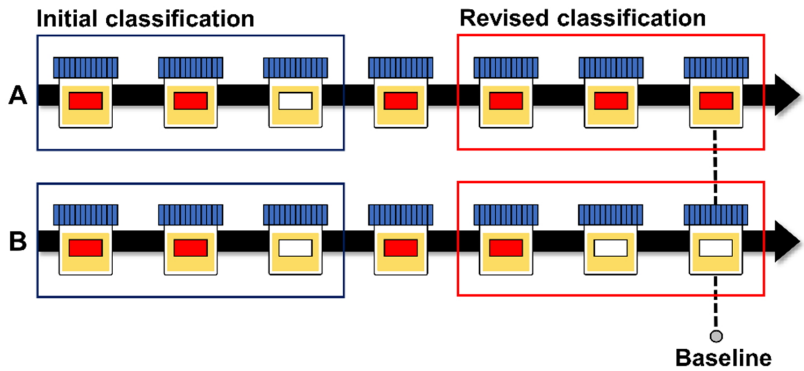


Figure 10 **A pictorial example to support the definition of albuminuria regression**. Timelines for two hypothetical study participants (A and B) are presented. Urine samples with white label represent moderate albuminuria, and urine samples with red label represent severe albuminuria. The rightmost samples are the 24-h urine collections at the first FinnDiane visit, that is, at the study baseline. The most advanced classification of albuminuria prebaseline (here, severe albuminuria for both A and B) is highlighted by the blue boxes. The revised classification (red boxes) is based only on the three most recent urine samples at the time point of the first FinnDiane visit. In the example, patient B would be categorized as a regressor because the revised classification (moderate albuminuria) is less advanced than the initial one (severe albuminuria).

5.2 Lipid and apolipoprotein determinations

The FinnDiane visit serum lipids and apolipoproteins were determined from non-fasting blood samples in the research laboratory of Professor Marja-Riitta Taskinen, Department of Medicine, Division of Cardiology, Helsinki University Central Hospital, Helsinki, Finland.

Serum total cholesterol and triglycerides were first measured enzymatically using a Cobas Mira analyzer (Hoffman-La Roche, Basel, Switzerland) with commercially available kits (Hoffman-La Roche until November 2001; ABX Diagnostics, Montpellier, France, until January 2006) and then with a Konelab 60i analyzer (Thermo Fischer Scientific, Waltham, MA, USA) with kits from the same manufacturer.

HDL-cholesterol concentration was determined with the HTS 7000 Plus Bio Assay Reader (Perkin Elmer, Waltham, MA, USA) with a commercial kit from Roche Diagnostic Hitachi (Hitachi, Tokyo, Japan).

Serum apoC-III was determined using an immunoassay method (Kamiya Biomedical Company, Tukwila, WA 98168, USA). ApoB concentration was determined with immunoassay (Orion Diagnostica, Espoo, Finland) until January 2006, and then with Konelab 60i analyzer and a commercial kit (Thermo Fischer Scientific, Waltham, MA, USA).

5.2.1 Calculation of LDL-cholesterol

Study IV calculated LDL-cholesterol (mmol/L) using the Friedewald formula [296], which operates at triglyceride concentrations <4.0 mmol/L. The equation introduced in 2020 by Sampson *et al.* [297] was utilized for LDL-cholesterol calculation in Studies II to III to be able to include participants with hypertriglyceridemia (up to 9.0 mmol/L). The equation has appeared more precise than previous ones. The LDL-cholesterol equation by Sampson *et al.* is based on measured total cholesterol, HDL-cholesterol, and triglyceride concentrations, as well as on calculated non-HDL-cholesterol and numerical constants.

5.2.2 Calculation of remnant cholesterol

Remnant cholesterol (mmol/L) was estimated using the following formula:

Remnant cholesterol = Total cholesterol – HDL-cholesterol – LDL-cholesterol

Thus, remnant cholesterol represents the cholesterol content of chylomicrons (minor contribution), chylomicron remnants, VLDL, and IDL particles in the non-fasting state.

5.2.3 Calculation of remnant cholesterol variability

All available lipid determinations retrieved from the medical files from the participating diabetes clinics were pooled with the measurements from the FinnDiane Study visits for the calculation of intraindividual remnant cholesterol variability. Remnant cholesterol calculations from the first FinnDiane visit until confirmed progression of DKD or the most recent date of sustained DKD states were included.

Variability was assessed for the study participants with remnant cholesterol calculations at three or more time points. Thus, 2,768 individuals with a median of 8 (range 3 to 43) remnant cholesterol calculations per person were studied.

Intraindividual variability was calculated using the following formula to correct for the strong interference of the mean on the standard deviation:

Coefficient of variation (CV) = standard deviation / intrapersonal mean

The CVs are presented as percentages in the results.

5.3 Additional clinical characteristics

The following clinical characteristics apply to Studies II-IV.

5.3.1 Pharmacotherapy

Two main classes of drugs were noted: 1) drugs inhibiting the RAAS, defined as the use of an ACE inhibitor and/or an ARB, and 2) lipid-lowering medication, defined as the use of statins, fibrates, ezetimibe, and/or a PCSK9 inhibitor, of which statins were the predominantly used therapeutic agents.

5.3.2 HbA_{1c}

HbA_{1c} was measured at local study centers with standardized assays, designating 20-42 mmol/mol (4.0-6.0%) as the normal range.

5.3.3 Blood pressure

Blood pressure was measured twice from the brachial artery in a sitting position after ten minutes of rest. The mean values for systolic and diastolic blood pressure were recorded.

5.3.4 Anthropometric measurements

Body weight was measured to the closest 0.1 kg with a standardized scale and height to the closest 1 cm with a wall-mounted stadiometer. Body-mass index (BMI) was calculated as weight (kg) divided by height (m) in square. The waist circumference was measured midway between the lowest rib and the iliac crest, and the hip circumference was measured at the widest part of the gluteal region. The waist-to-hip ratio (WHR) was determined as the waist circumference divided by the hip circumference.

5.3.5 History of smoking

Former and current smoking habits were assessed at the FinnDiane visit using a self-administered questionnaire. The smoking status was designated *current* if the participant had a recurrent habit of smoking at least one cigarette per day at the time of the visit. The smoking status was labeled *former* if the participant had ceased a frequent habit of smoking (at least one cigarette per day for three months or more) before entering the study.

5.4 Cardiovascular events

Data on cardiovascular events were retrieved from the Care Register for Health Care and the Finnish Causes of Death Register (fatal events). The cardiovascular outcome of interest, henceforth referred to as CVD, was a composite endpoint including the first occurring of acute myocardial infarction (ICD-8/9: 410–412, ICD-10: I21–I23; www.who.int/classifications/icd/en/), cerebrovascular accident (ICD-8/9: 430–434, ICD-10: I60–I64), and coronary procedure (bypass grafting surgery or angioplasty based on the Nordic Classifications of Surgical Procedure codes TFN40, FN1AT, FN1BT, FN1YT, FNF, FNG, FNA, FNB, FNC, FND, FNE). Both fatal and non-fatal events were considered. CVD events were obtained through 31 December 2017 in Studies II and III and 31 December 2014 in Study IV.

5.5 Mortality

Data on mortality were retrieved from the Finnish Causes of Death Register, which is maintained by Statistics Finland. Mortality data were obtained through 31 December 2017 in Studies II and III and 31 December 2014 in Study IV. Vital status was checked from the medical records in Study I.

5.6 Statistical analyses

Variable distributions were assessed graphically. Differences between continuous variables with symmetric distributions were analyzed with Student's t-test (two groups) or ANOVA (more than two groups) and are presented as mean ± standard deviation. Continuous variables with skewed distributions were analyzed with the Mann–Whitney U test (two groups) or Kruskal–Wallis test (more than two groups) and are presented as median (IQR, *i.e.,* from the 25th to the 75th percentile). Between-group differences for categorical variables were analyzed with Pearson's χ^2-test and are presented as n (%). Significance was taken at $p<0.05$ (two-sided p-value).

Study I split each study participant's observation time into multiple observations by 0.5-year intervals of diabetes duration (*i.e.,* since the onset of diabetes) using the Lexis macro in SAS [298]. The split data were used to assess duration-specific incidences of moderate and severe albuminuria. First, patterns of the duration-specific incidences were evaluated by fitting generalized additive models (GAM) [299] to the split data without *a priori* assumptions of the shape of the relationship between diabetes duration and the albuminuria incidence rate. Thereafter, the split data were grouped into five-year intervals and incidence rates calculated for each interval. Mean incidence rates were calculated by dividing the number of events by accumulated person-years. Duration-specific incidence rates were calculated separately for the 1970-79 and 1980-99 diabetes diagnosis calendar-year cohorts, and Poisson regression models were used to evaluate differences between these. GAM modelling was also used to evaluate the relationship between age at diabetes onset and severe albuminuria risk.

Multivariable Cox proportional hazards regression analyses were performed in every study included in this thesis. Study I utilized Cox regression to determine the association between clinical characteristics (diabetes diagnosis year, age at diabetes onset, and sex) and the risk of albuminuria in the cohort. The onset age was categorized into three groups (0-4 years, 5-9 years, and 10-14 years) because of the nonlinear relationship between the risk of DKD and the age at diabetes onset revealed by the GAM modeling. Studies II-IV used Cox regression to assess the relationship between remnant cholesterol, apoC-III, and albuminuria regression, respectively, and the endpoints of interest (progression of DKD, CVD, and mortality). These analyses were adjusted stepwise for potential non-modifiable and modifiable covariates. Covariates with skewed distributions were log-transformed. BMI was included as restricted cubic splines with three knots based on Akaike information criterion minimization to reduce overfitting, due to its previously shown nonlinear relationship with DKD [152]. Results from Cox proportional hazards regression analyses are presented as HRs with 95% CIs. The variance inflation factor was checked at each step of regression modeling to detect multicollinearity. The factors did not exceed 3 in any of the models used. Study

IV assessed associations between clinical variables and the regression of albuminuria using logistic regression analysis.

The Kaplan-Meier estimator was used to portray time-to-event and to calculate cumulative incidences in this thesis. The log-rank test was used to assess between-group differences in risk. The Kaplan-Meier curves were stratified by the decade of diabetes diagnosis, sex, and age at diabetes onset in Study I and by albuminuria status (including albuminuria regression) in Study IV. The Kaplan-Meier analysis was also used in Study I to illustrate and calculate the cumulative progression rates from moderate to severe albuminuria. The progression from severe albuminuria to kidney failure was defined by the Fine and Gray subdistribution hazard model, including death as a competing risk [300].

Data were analyzed with the R open-source software version 4.1.1 (Studies I-IV), SAS version 9.4 (Study I), and IBM SPSS Statistics for Windows version 22.0 (Study IV).

6 RESULTS

Results from the original publications (Studies I-IV) and some previously unpublished data are presented next.

6.1 Incidence of diabetic kidney disease (Study I)

6.1.1 Initiation and progression of diabetic kidney disease

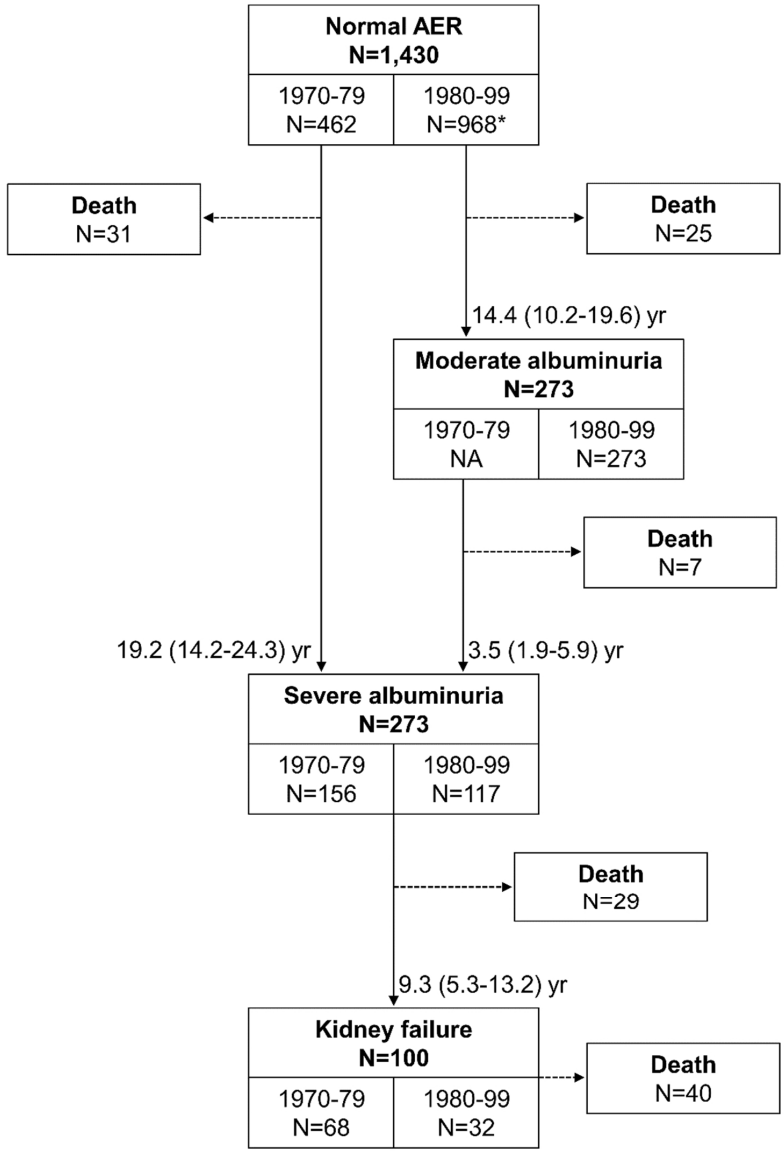
The flowchart in Figure 11 provides an overview of the development and progression of DKD in Study I.

Among the individuals with diabetes since 1980-99, 28.4% (n=273) developed moderate albuminuria. The median time from onset of diabetes to moderate albuminuria was 14.4 (IQR 10.2-19.6) years. Of these individuals, 117 further developed severe albuminuria, with a median time of 3.5 (IQR 1.9-5.9; range 0.23-19.9) years between the two albuminuria stages. The 5-year cumulative progression rate from moderate to severe albuminuria was 32.2% (95% CI 26.1-37.9) and the 15-year progression rate was 54.3% (46.0-61.3). The cumulative progression from moderate to severe albuminuria appears in Figure 12a.

Among the individuals with diabetes onset in 1970-79, 33.8% (n=156) developed severe albuminuria. The median time from the onset of diabetes to a diagnosis of severe albuminuria was 19.2 (14.2-24.3) years in the 1970-79 cohort.

Moreover, 7.0% of the cohort progressed to a need for kidney replacement therapy during the follow-up. The 15-year cumulative progression rate from severe albuminuria to kidney failure was 35.2% (95% CI 27.4-43.0) in the 1970-79 calendar-year diagnosis cohort after the onset of severe albuminuria. Of note, the cumulative progression was no different compared to the two recent calendar-year cohorts combined (1970-70 vs. 1980-99, Gray's test p=0.37), the latter group reaching a progression rate of 35.6% (24.3-47.0) 15 years after severe albuminuria had been diagnosed.

Figure 11 **The development of diabetic kidney disease in Study I.** Progression times between the different stages of diabetic kidney disease are given as median (interquartile range) years. *Data on moderate albuminuria were available altogether in 961 individuals from the 1980-99 cohort, whereas data on severe albuminuria were available for 968 individuals. Copyright © 2022 Elsevier Ltd. Adapted from Study I (Figure 1) with permission from the copyright holder.



Mortality was a competing risk for DKD, particularly at the later stages of kidney disease (Figure 11). Of the 100 individuals who progressed to kidney failure, 40 died during follow-up. Figure 12b presents the cumulative progression from severe albuminuria to kidney failure by diagnosis cohort, incorporating death as a competing risk.

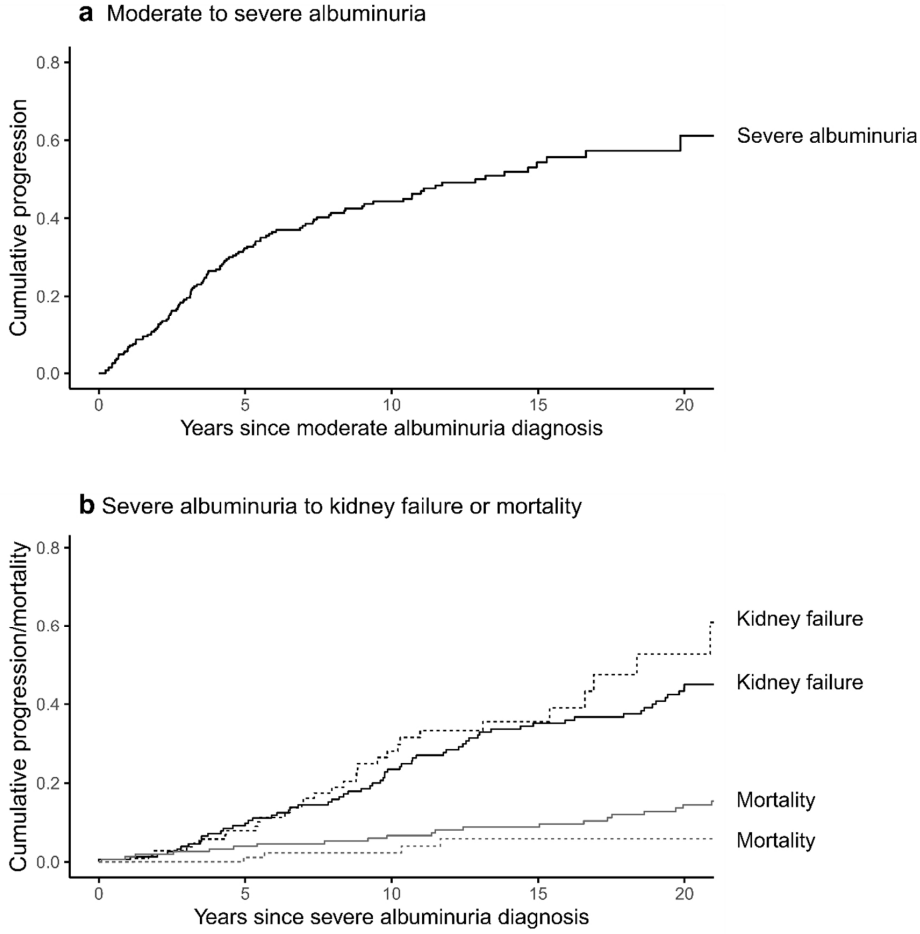


Figure 12 **The cumulative progression from moderate to severe albuminuria (a) and severe albuminuria to kidney failure (b).** In figure **b**, the competing risk of mortality was accounted for. In figure **b**: solid line, 1970-79; dashed line, 1980-99. Copyright © 2022 Elsevier Ltd. Adapted from Study I (Figure 4) with permission from the copyright holder.

6.1.2 Cumulative incidence of moderate albuminuria

Figure 13 displays the cumulative incidence of moderate albuminuria stratified by calendar-year diagnosis cohorts of diabetes diagnosis. No difference was noted between the two assessed cohorts: The HR for moderate albuminuria was 0.99 (95% CI 0.78-1.28) with the 1980-89 cohort as reference, p=0.97. After 15 years of diabetes, 15.5% (95% CI 12.2-18.7) of the 1980-89 cohort had developed moderate albuminuria, and the corresponding proportion was 15.6% (12.2-18.8) in the 1990-99 cohort. The cumulative incidences after 25 years of diabetes were 29.8% (25.4-33.9) and 30.7% (24.8-36.2), respectively.

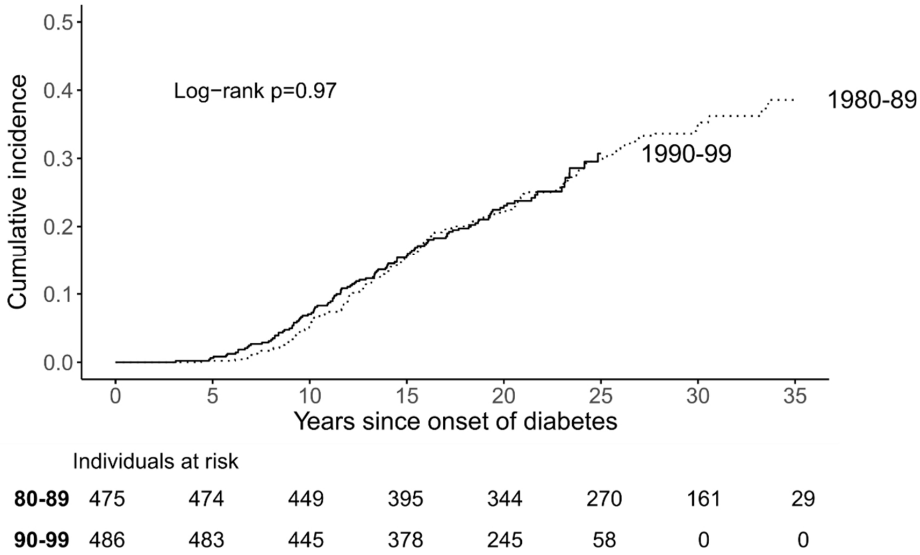


Figure 13 **The cumulative incidence rates of moderate albuminuria by the calendar-year cohort of a diabetes diagnosis**. Dotted line, diagnosis cohort 1980-89; solid line, diagnosis cohort 1990-99. Copyright © 2022 Elsevier Ltd. Adapted from Study I (Figure 3A) with permission from the copyright holder.

6.1.3 Cumulative incidence of severe albuminuria

Figure 14 portrays the cumulative incidences of severe albuminuria by calendar-year diagnosis cohort. A large drop between the 1970-79 cohort and the two later ones was observed: With the 1970-79 cohort as reference, the HR was 0.55 (95% CI 0.42-0.72), p<0.001, for the 1980-89 diagnosis cohort and 0.36 (95% CI 0.25-0.53), p<0.001, for the 1990-99 diagnosis cohort. However, there was no difference between the two latter diagnosis cohorts, because the HR for pair-wise comparison was 0.83 (95% CI 0.54-1.26) with 1980-89 as

reference, p=0.38. The calculated 25-year cumulative incidence rates were 26.8% (95% CI 22.6-30.8), 12.0% (9.0-15.0) and 10.8% (6.7-14.6) for the 1970-79, 1980-89, and 1990-99 cohorts, respectively. After 35 years of diabetes, 32.6% (28.1-36.9) of the earliest diagnosis cohort (1970-79) had developed severe albuminuria and 37.8% (32.6-42.6) after 45 years. The study subjects in the 1980-89 and 1990-99 diagnosis cohorts were combined in some of the following analyses on severe albuminuria due to the similar cumulative incidence rates and the limited number of individuals in the 1990-99 cohort later in the follow-up.

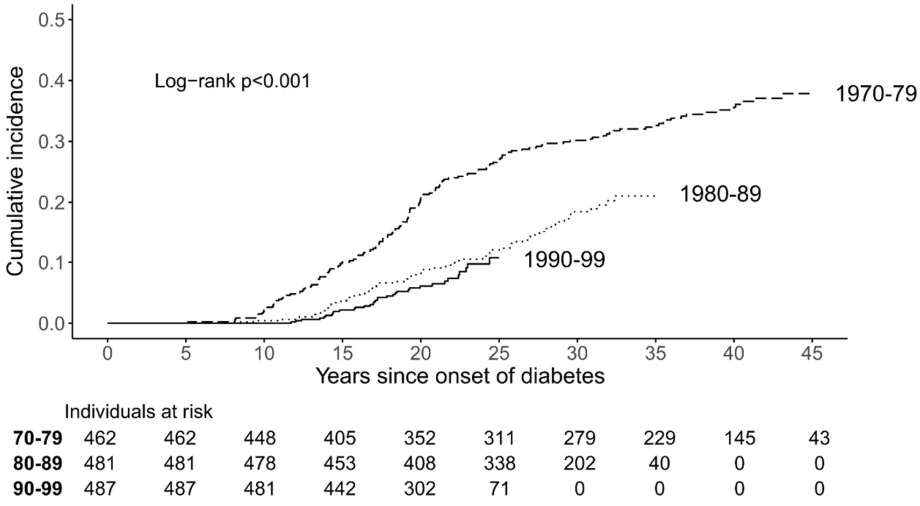


Figure 14 **The cumulative incidence of severe albuminuria by the calendar-year cohort of a diabetes diagnosis.** Dashed line, diagnosis cohort 1970-79; dotted line, diagnosis cohort 1980-89; solid line, diagnosis cohort 1990-99.

6.1.4 Duration-specific incidence rates of moderate albuminuria

We next assessed the incidence rates of moderate albuminuria according to the duration of diabetes. The smoothing plot (Figure 15a) shows that the incidence rose significantly until ten years of diabetes duration, whereafter it remained rather stable until it started to decrease slightly at around 25 years of diabetes. The mean incidence rate during the plateau phase (10 to 24 years of duration) was 19.2 (16.5-22.2) cases per 1,000 person-years. Incidence rates by 5-year intervals of diabetes duration appear in Figure 15b.

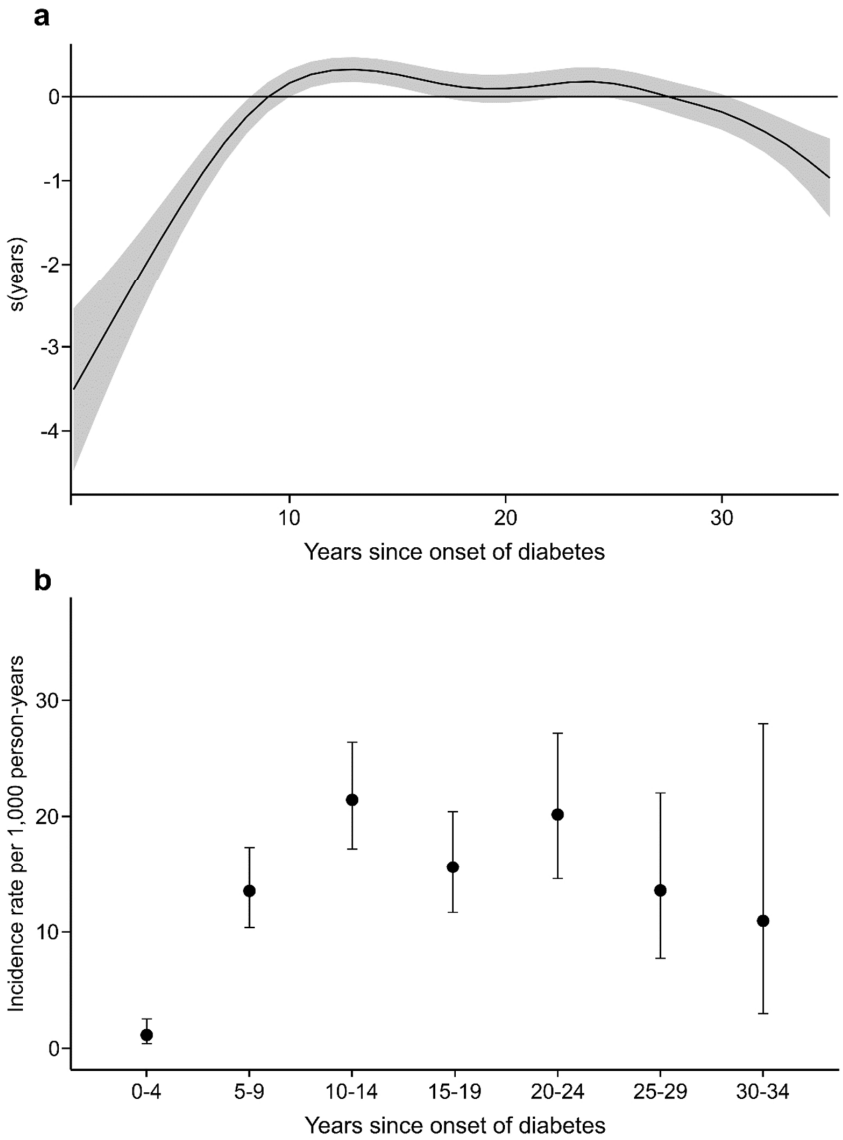
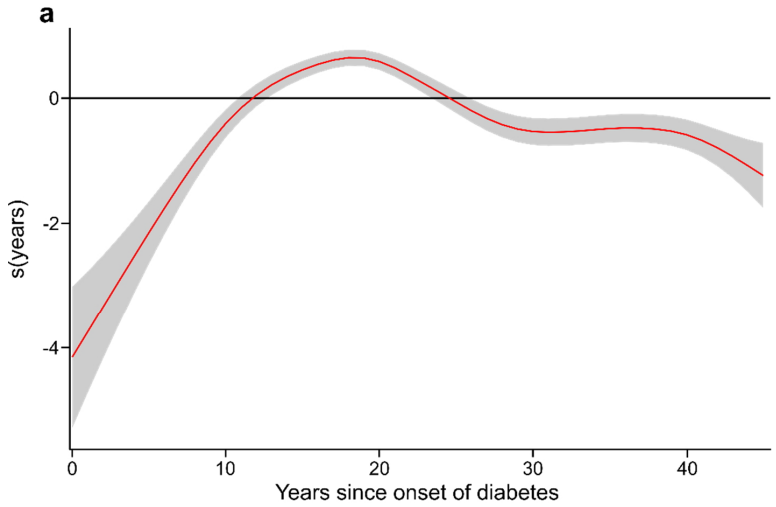


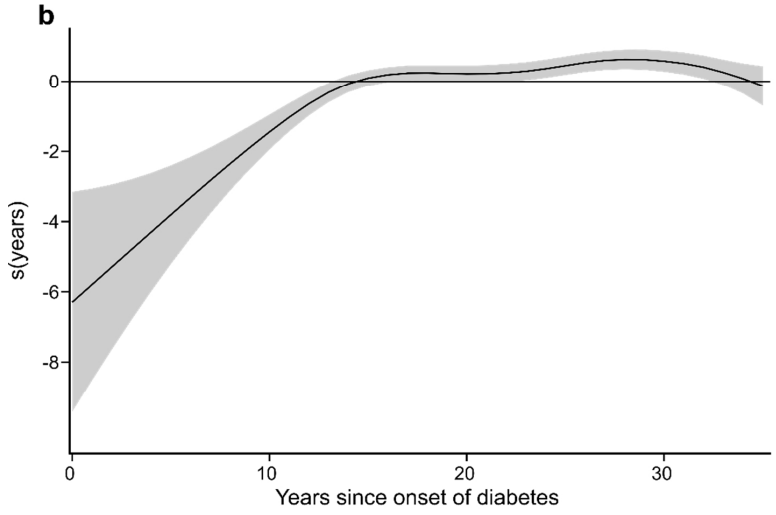
Figure 15 **Diabetes duration-specific incidence rates of moderate albuminuria.** Figure **a** shows the smooth function resulting from GAM modelling for the annual incidence of moderate albuminuria. The grey area denotes the 95% confidence interval. Figure **b** depicts the incidence rates per 5-year intervals of diabetes duration with 95% confidence intervals.

6.1.5 Duration-specific incidence rates of severe albuminuria

The incidence pattern of severe albuminuria had changed between the 1970-79 and 1980-99 cohorts (p for interaction <0.001). The incidence rate peaked 15-19 years after the onset of diabetes (at 25.8 [19.1-34.1] per 1000 person-years) in the 1970-79 calendar-year cohort, whereafter it dropped and remained at a lower level throughout the observed period. In the more recent calendar-year cohorts, the incidence rate upsurge leveled out after 14 years of diabetes. Thereafter, the incidence was on average 10.2 (8.2-12.6) cases per 1000 person-years and, of note, it did not decrease during the observation period. The severe-albuminuria incidence rate ratios for the 1980-99 cohort (1970-79 as reference; p-values denote between-group differences) were 0.10 at 5-9 years (p<0.001), 0.35 at 10-14 years (p<0.001), 0.38 at 15-19 years (p<0.001), 0.52 at 20-24 years (p=0.01), 2.27 at 25-29 years (p=0.04), and 1.11 at 30-34 years of diabetes (p=0.85). The smoothing plots and incidence rate plots for severe albuminuria are shown in Figures 16a-c.

Figure 16 **Diabetes-duration specific incidence rates of severe albuminuria** (*next page*). Figures **a** and **b** show the smooth functions from GAM modelling for the annual incidences of severe albuminuria in the 1970-70 and 1980-99 cohorts, respectively. The grey areas denote the 95% confidence intervals. Figure **c** depicts the incidence rates per 5-year intervals of diabetes duration with 95% confidence intervals.


a
0
-2
-4
s(years)
0
10
20
30
40
Years since onset of diabetes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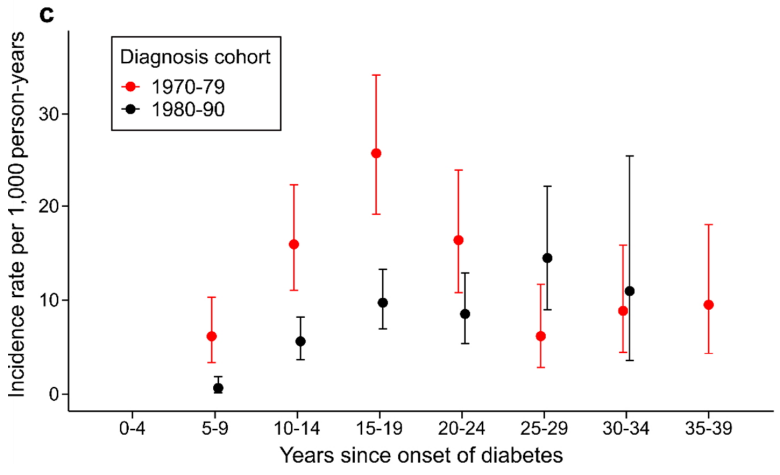
Years since onset of diabetes


c
Diagnosis cohort
1970-79
1980-90
Incidence rate per 1,000 person-years
0
10
20
30
0-4
5-9
10-14
15-19
20-24
25-29
30-34
35-39
Years since onset of diabetes

6.1.6 Sex, age at diabetes onset, and the incidence of albuminuria

The GAM modeling revealed a peak in the risk of severe albuminuria when the age at onset of diabetes was between nine and ten years (Figure 17). The overall lowest cumulative incidence rate of DKD was observed among those with early onset of diabetes (0-4 years; Figure 18) when age at onset was categorized into three groups (0-4, 5-9, and 10-14 years). The 5-9 and 10-14 age at onset categories showed comparable risks (log-rank p=0.47 and 0.32 between these two categories for moderate and severe albuminuria, respectively). Women and men were no different concerning either of the albuminuria categories with all individuals combined. Nevertheless, the interaction term between the age at onset categories and sex was significant (p=0.04), indicating a sex difference that is dependent on the age at onset.

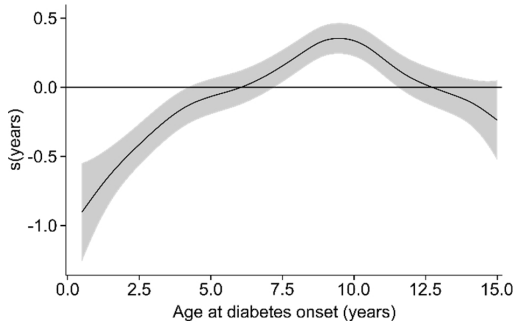
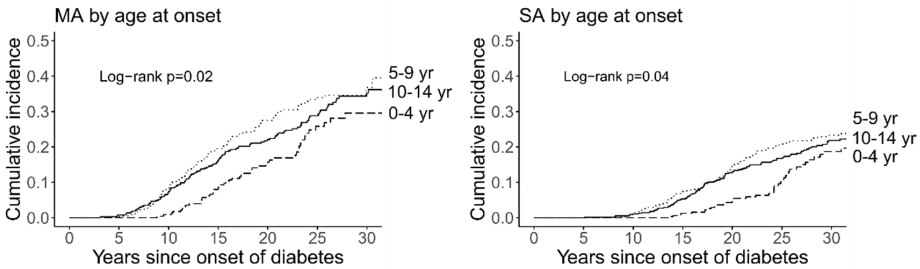


Figure 17 Smooth function for the interaction between the risk of severe albuminuria and the age at diabetes onset. The grey area denotes the 95% confidence interval.

Therefore, we assessed differences between the sexes within age at diabetes onset categories separately. The cumulative incidences of moderate and severe albuminuria from these analyses are presented in Figure 18. The only sex difference in the stratified analyses was noted among those with diabetes since the age of 10-14 years (HR 1.53 [95% CI 1.04-2.26)] p=0.03; women as reference). The HR for men in the category with diabetes onset at 0-4 years was 1.46 (0.83-2.58), p=0.18, and 0.82 (0.56-1.18), p=0.28, in the 5-9-year category. The same phenomenon was observed for severe albuminuria: The HRs for men (women as reference) were 1.19 (0.66-2.15; p=0.56), 0.96 (0.67-1.38; p=0.82) and 1.75 (1.20-2.55; p=0.004) for the age at onset categories 0-4, 5-9, and 10-14, respectively.



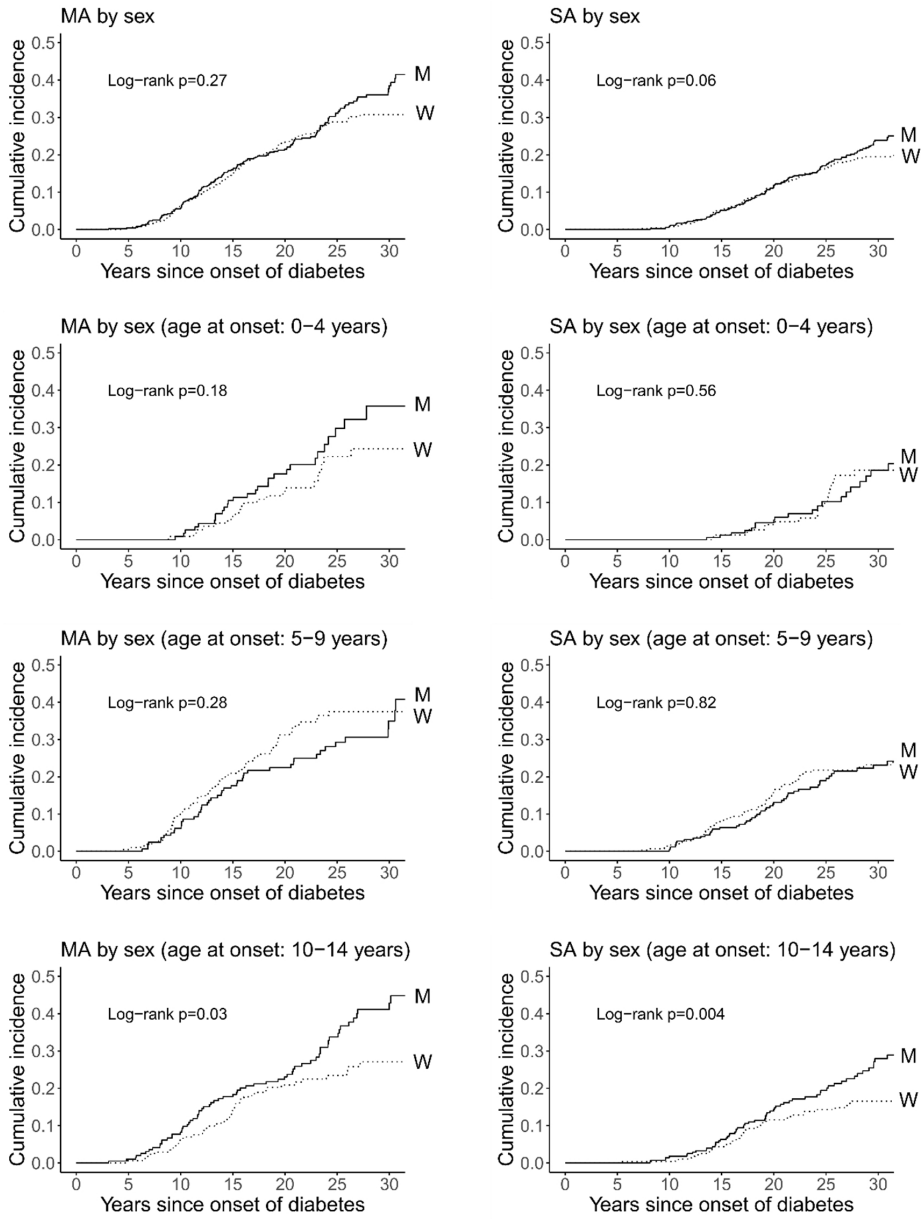


Figure 18 **The cumulative incidence rates of moderate and severe albuminuria with stratification for the category of age at diabetes onset and sex** (*continuation from the previous page*). MA, moderate albuminuria; SA, severe albuminuria; yr, years; M, men; W, women. "Age at onset" refers to the age when type 1 diabetes was diagnosed.

We subsequently aimed to investigate the impact of the available clinical characteristics (calendar year of diabetes diagnosis, age at diabetes onset, and sex) on the risk of moderate/severe albuminuria with a multivariable Cox regression analysis. These analyses included calendar year at diabetes onset as a continuous variable.

Table 4 presents the results of the analysis. The calendar year of diabetes diagnosis was significantly associated with severe albuminuria: The adjusted hazard of severe albuminuria decreased by 5% (HR 0.95 [0.93-0.97], p<0.001) for every one-year increment in the calendar year. Furthermore, diabetes debut at the age of 5-9 conferred an independently higher risk of both moderate and severe albuminuria than the 0-4-year reference group. Despite the univariate differences in risk between the 10-14 and 0-4-year groups, there was no difference between these after sex was included as a covariate in the multivariable analyses.

Table 4 The association between clinical characteristics and moderate/severe albuminuria: results of a multivariable Cox regression analysis.

	Moderate albuminuria		Severe albuminuria	
Individuals	*n=961*		*n=1,430*	
Events	*n=273*		*n=273*	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Diagnosis year	0.99 (0.96-1.01)	0.21	0.95 (0.93-0.97)	<0.001
Age at diabetes onset				
0-4 years	1.00 (reference)		1.00 (reference)	
5-9 years	1.60 (1.14-2.23)	0.006	1.48 (1.05-2.09)	0.03
10-14 years	1.38 (0.98-1.93)	0.07	1.32 (0.93-1.86)	0.12
Sex				
Women	1.00 (reference)		1.00 (reference)	
Men	0.99 (0.91-1.46)	0.25	1.27 (1.00-1.61)	0.05

6.1.7 Survival after the onset of albuminuria

Table 5 presents mortality rates after the onset of moderate and severe albuminuria (previously unpublished data, Jansson Sigfrids *et al.*). Fifteen years after the onset of moderate albuminuria, the cumulative mortality rate was 8.8%, whereas the corresponding rate regarding severe albuminuria was 15.3%. We saw no improvement in the survival (log-rank p=0.55) when those with diabetes since 1970-79 were compared with the 1980-99 onset cohort (1980-89 and 1990-99 were combined).

Of the 69 individuals who had been diagnosed with severe albuminuria before their death, 40 (58%) had further advanced to kidney failure. Three main groups of causes of death were identified among the 69 subjects: 1) cardiovascular diseases – 46% of the deaths (72% of these were due to acute myocardial infarctions, 19% due to strokes, the rest due to other cardiac diseases); 2) multiple chronic complications of diabetes, together with a fatal infection (often pneumonia) – 17% of the deaths; 3) multiple chronic complications of diabetes with no further specification – 17% of the deaths. All but two persons in the third group received kidney replacement therapy. Deaths due to acute diabetes complications, neoplasms, and "risky behavior" (alcohol misuse, intentional self-harming, *etc.*) occurred but were rare.

Table 5 Cumulative mortality rates after the onset of moderate and severe albuminuria in Study I.

	Number at risk	Cumulative number of events	Cumulative mortality rate (95% confidence interval)
After the onset of moderate albuminuria			
5 years	237	5	2.0% (0.2-3.6)
15 years	100	17	8.8% (4.5-12.9)
After the onset of severe albuminuria			
All individuals (1970-99)			
5 years	230	10	4.0% (1.5-6.4)
15 years	131	33	15.3% (10.3-20.0)
1970-70			
5 years	143	8	5.2% (1.6-8.7)
15 years	107	22	15.1% (9.0-20.7)
1980-99			
5 years	87	2	2.1% (0.0-5.0)
15 years	24	11	17.1% (6.9-26.2)

6.2 Remnant cholesterol and apolipoprotein C-III: cross-sectional analyses (Studies II-III)

6.2.1 Remnant cholesterol

Figure 19 presents the concentrations of remnant cholesterol (mmol/L) stratified by some relevant clinical characteristics. Median concentrations of remnant cholesterol were higher among men and current/former smokers and increased with increasing BMI category. Furthermore, we noted a positive correlation between remnant cholesterol and HbA_{1c} ($r=0.21$, $p<0.001$), as well as between remnant cholesterol and systolic blood pressure ($r=0.14$, $p<0.001$). The correlation between remnant cholesterol and diabetes duration was non-significant despite the significant difference when stratifying by the median diabetes duration. The correlation between remnant cholesterol and LDL-cholesterol was $r=0.22$, $p<0.001$.

6.2.2 Apolipoprotein C-III

A positive correlation between the concentrations of baseline remnant cholesterol and apoC-III (mg/dL) was observed: The correlation coefficient was 0.58, $p<0.001$. The correlation between apoC-III and LDL-cholesterol was 0.13, $p<0.001$. The apoC-III concentration in relation to relevant clinical characteristics appears in Figure 20. The finding was quite analogous to what was just noted for remnant cholesterol. The correlations between apoC-III and HbA_{1c} and apoC-III and systolic blood pressure were both 0.16 ($p<0.001$). The correlation between apoC-III and diabetes duration was not significant in accordance with the finding for remnant cholesterol.

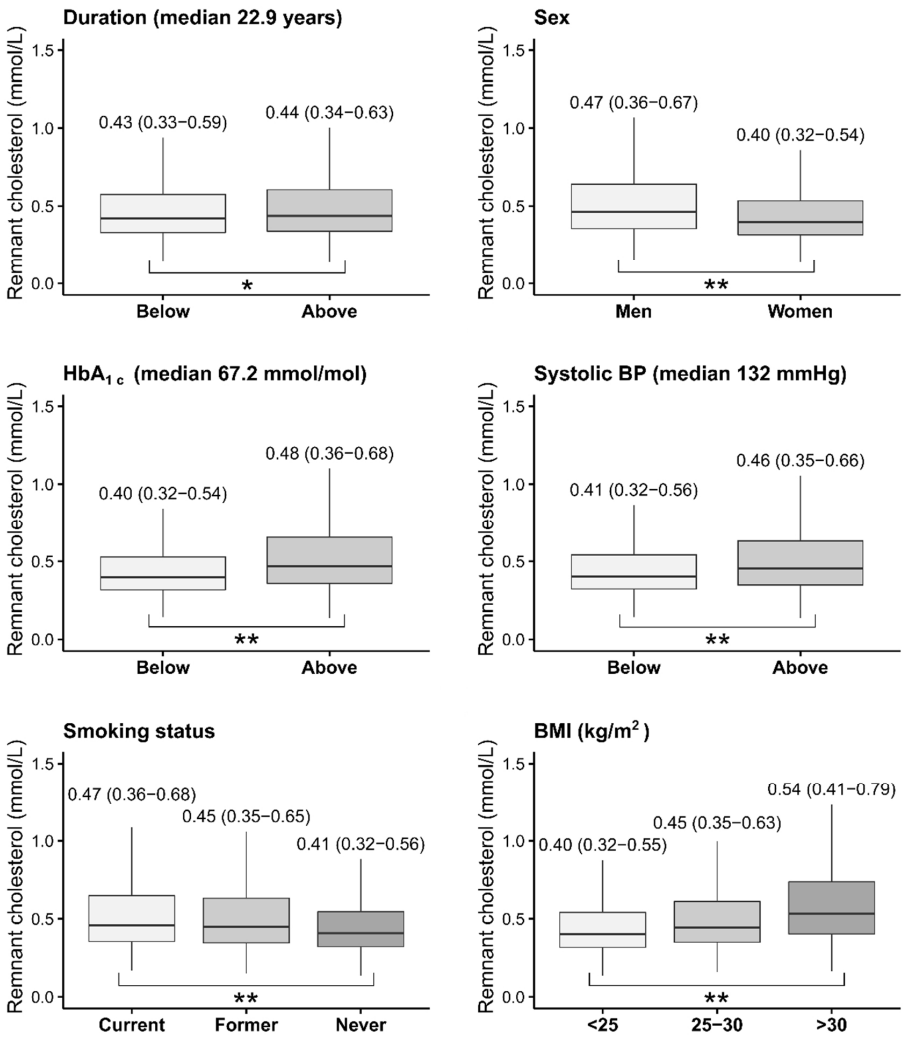


Figure 19 **Remnant cholesterol concentration in relation to relevant clinical characteristics at baseline.** The boxes indicate median with interquartile range (denoted also in text above). The whiskers indicate the range (excluding potential outliers). For diabetes duration, HbA_{1c}, and systolic blood pressure, the cohort was stratified by the median of each variable, which is indicated in the title of each figure. BP, blood pressure. *, p<0.05; **, p<0.001.

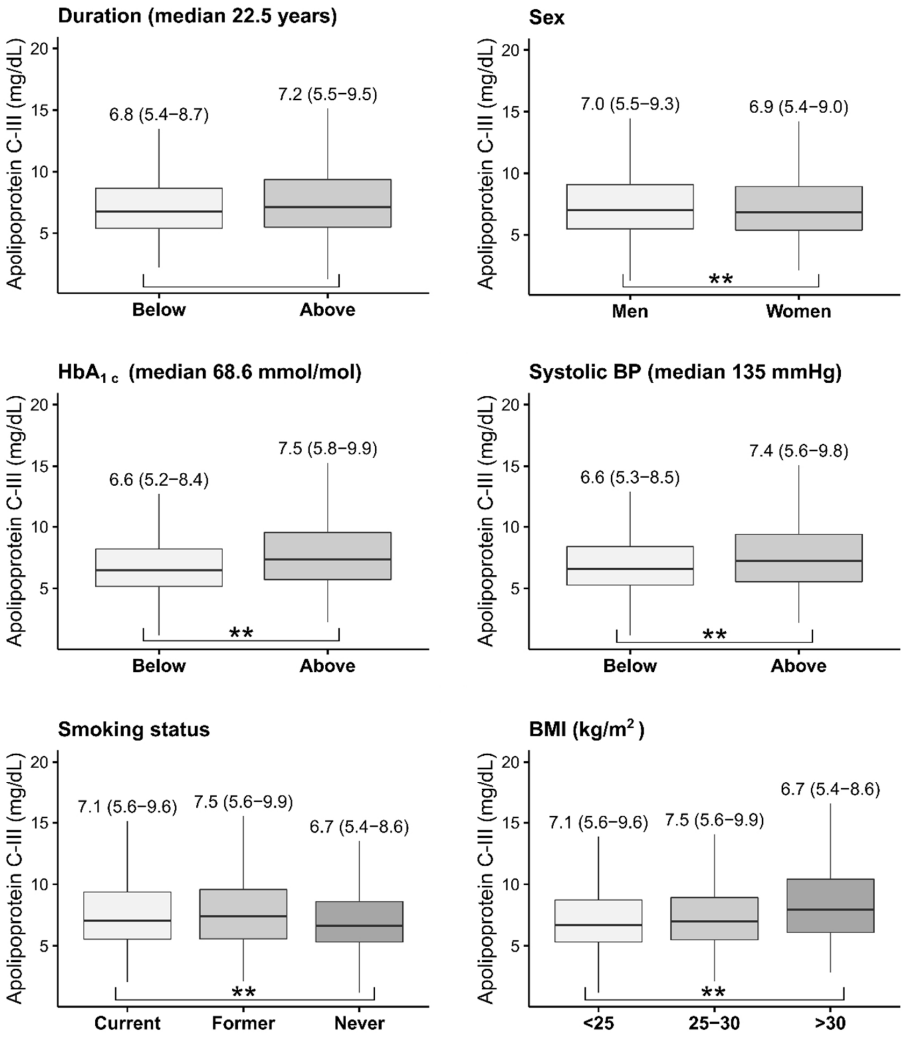


Figure 20 **ApoC-III concentration in relation to relevant clinical characteristics at baseline.** The boxes indicate median with interquartile range (denoted also in text above). The whiskers indicate the range (excluding potential outliers). For diabetes duration, HbA_{1c}, and systolic blood pressure, the cohort was stratified by the median of each variable, which is indicated in the title of each figure. BP, blood pressure. *, p<0.05; **, p<0.001.

6.2.3 Association with diabetic kidney disease (cross-sectional analyses)

Our analyses revealed that the baseline concentration of remnant cholesterol increased with advancing category of kidney disease (Figure 21), the step between severe albuminuria and kidney failure being an exception. The concentrations within the kidney failure group were lower in those who had received a kidney transplant (0.57 [0.42–0.74] mmol/L) than those who had not (0.63 [0.46–0.96] mmol/L), p=0.008. The correlation between remnant cholesterol and eGFR was r=-0.21, p<0.001.

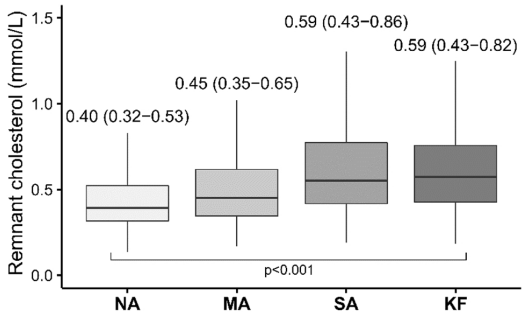


Figure 21 **Remnant cholesterol concentration in relation to DKD stage at baseline**. The boxes indicate median with interquartile range (denoted also in text above). The whiskers indicate the range (excluding potential outliers). NA, normal albumin excretion rate; MA, moderate albuminuria; SA, severe albuminuria; KF, kidney failure.

Along similar lines, the apoC-III concentration increased with the baseline DKD stage (Figure 22). There was a significant correlation (p<0.001) between apoC-III and eGFR, r=-0.32.

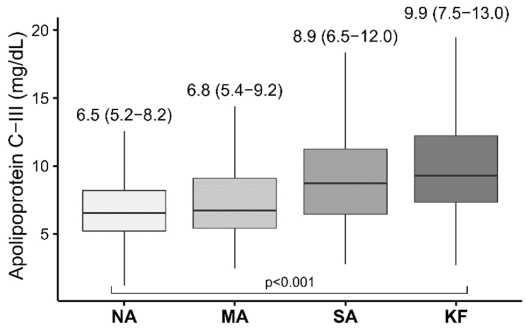


Figure 22 **ApoC-III concentration in relation to DKD stage at baseline.** The boxes indicate median with interquartile range (denoted also in text above). The whiskers indicate the range (excluding potential outliers). NA, normal albumin excretion rate; MA, moderate albuminuria; SA, severe albuminuria; KF, kidney failure.

6.2.4 Association with the use of lipid-lowering medication

Among the study participants in Study II, 787 (16%) were using a lipid-lowering agent at baseline. Not surprisingly, this proportion increased with the advancing stage of DKD, ranging from 9% in the group of normal AER to 42% in the kidney failure group (p for trend <0.001). The median (IQR) remnant cholesterol concentration was 0.52 (0.40-0.72) mmol/L among the study subjects who used a lipid-lowering agent and 0.42 (0.33-0.59) mmol/L among those who did not, p<0.001 (Figure 23).

Among the study participants in Study III, 729 (19%) were on lipid-lowering treatment at baseline. This proportion also increased with the baseline DKD stage, ranging from 10% in the group of normal AER to 49% in those with kidney failure (p<0.001 for trend). The median apoC-III concentration was 8.4 (6.2-11.0) mg/dL among the study subjects who used a lipid-lowering agent and 6.7 (5.4-8.6) mg/dL among those who did not, p<0.001 (Figure 23).

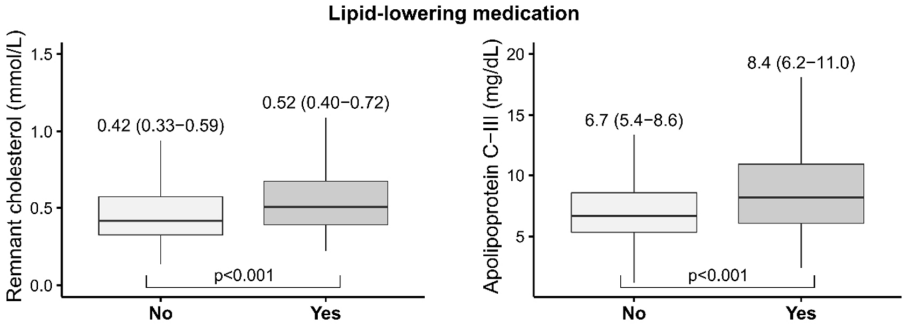


Figure 23 **Remnant cholesterol and apoC-III concentration in relation to the use of lipid-lowering medication at baseline.** The boxes indicate median with interquartile range (also denoted in text above). The whiskers indicate the range (excluding potential outliers).

6.3 Progression of diabetic kidney disease (Studies II-III)

6.3.1 Remnant cholesterol and apolipoprotein C-III concentration

The DKD stage of 642 (16.6%) study participants progressed in Study II; 266 (9.9%) from normal AER to moderate albuminuria, 110 (20.2%) from moderate to severe albuminuria, and 266 (41.0%) from severe albuminuria to kidney failure. The median follow-up time was 8.0 years (IQR 4.9-13.7) and 35,226 person-years in total. The proportion of progressors was slightly lower in Study III due to the previously described differences in cohort compilations: The DKD stage of 14.6%, corresponding to 451 individuals, progressed during the median follow-up period of 7.6 years (4.4-12.2).

Table 6 presents clinical characteristics stratified by DKD progression status (the cohort in Study II). A larger proportion of the progressors were men, the progressors were older, and they had a longer diabetes duration. Moreover, the progressors were distinguished by a poorer glycemic control and a worse lipid profile than the nonprogressors.

Table 6 Clinical characteristics at the baseline FinnDiane visit stratified by diabetic kidney disease progression status.

	No progression	Progression	p-value
	n=3,237	*n=642*	
Age (years)	37.1±12.2	38.6±11.8	0.003
Age at diabetes onset (years)	14.8 (9.5-23.7)	12.8 (8.5-20.1)	<0.001
Diabetes duration (years)	20.4±12.2	23.7±11.3	<0.001
Sex (women)	1631 (50%)	259 (40%)	<0.001
HbA_{1c} (mmol/mol)	66.9±15.1	78.3±18.3	<0.001
Systolic blood pressure (mmHg)	132±17	140±21	<0.001
Diastolic blood pressure (mmHg)	79±9	82±11	<0.001
History of smoking			<0.001
Current	690 (22%)	205 (34%)	
Former	638 (21%)	162 (27%)	
Never	1758 (57%)	245 (40%)	
BMI (kg/m^2)	25.1±3.6	25.6±4.1	0.007
Total cholesterol (mmol/L)	4.83±0.90	5.23±1.15	<0.001
HDL-cholesterol (mmol/L)	1.37±0.38	1.25±0.42	<0.001
LDL-cholesterol (mmol/L)	2.98±0.83	3.26±0.99	<0.001
Triglycerides (mmol/L)	0.97 (0.74-1.34)	1.33 (0.95-2.08)	<0.001
eGFR (mL/min per 1.73 m^2)	104 (88-117)	86 (40-111)	<0.001
Use of RAAS inhibitor	843 (26%)	347 (55%)	<0.001
Use of lipid-lowering agent	288 (9%)	136 (21%)	<0.001

Data are mean ± standard deviation, median (interquartile range), or n (%).

Remnant cholesterol also differed by DKD progression status: Its median concentration was 0.55 mmol/L (0.40–0.85) among the progressors and 0.41 mmol/L (0.32–0.55) among those who did not progress, p<0.001. Of note, the direction of difference (higher among progressors) and level of significance (p<0.001) applied to all stages of DKD progression. Along similar lines, the concentration of apoC-III was higher among the FinnDiane study participants whose DKD status progressed (8.1 mg/dL [6.1-10.9]) than those whose DKD status did not change (6.6 mg/dL [5.2-8.3]), p<0.001. This feature was also true for all stages of DKD progression.

We next wanted to study the association between remnant cholesterol/apoC-III and the progression of DKD in more detail; therefore, we performed Cox regression analyses with different levels of adjustment. The first stage was unadjusted; the second included the nonmodifiable covariates sex and diabetes duration; the third stage included sex and diabetes duration together with systolic blood pressure, HbA_{1c}, smoking status, and BMI – that is, modifiable covariates and known risk factors for DKD; eGFR was added to the model at the fourth stage; and remnant cholesterol and apoC-III were adjusted for each other at the fifth stage.

These analyses, whose results Table 7 presents, revealed the following: First, remnant cholesterol was robustly associated with the progression of DKD at all stages, independently of several modifiable and non-modifiable traditional DKD risk factors, as well as of apoC-III. The only exception was the last stage of progression (severe albuminuria to kidney failure), at which the association was lost after adjustment for eGFR. However, removing eGFR from the final model restored the association between remnant cholesterol and progression to kidney failure (HR 2.59 [95% CI 1.55-4.32], p<0.001), whereas the association between apoC-III and progression to kidney failure did not reappear (HR 1.40 [0.93-2.07], p=0.11). Second, the association between apoC-III and the progression from normal AER to moderate albuminuria lost significance after the addition of modifiable covariates to the model. Adjustment for the modifiable variables one at a time uncovered that inclusion of HbA_{1c} to the model was the step that resulted in a nonsignificant association between apoC-III and the outcome (p=0.27). The opposite was observed when the other covariates were included individually (systolic blood pressure as a covariate, p=0.049 for the association between apoC-III and the outcome; smoking status, p=0.02; BMI, p=0.003). Third, when progression from moderate albuminuria served as the outcome, apoC-III was robustly associated with the outcome, and the association was lost only when adjusting for remnant cholesterol. Fourth, when progression from severe albuminuria served as the outcome, adjustment for eGFR led to a nonsignificant finding between apoC-III and the outcome.

Table 7 The association between remnant cholesterol/apoC-III and the progression of diabetic kidney disease: results from multivariable Cox proportional hazards regression analyses.

	Remnant cholesterol		ApoC-III	
	HR (95% CI)	p-value	HR (95% CI)	p-value
All stages of progression combined				
Individuals	*n=3,879*		*n=3,085*	
Events	*n=642*		*n=451*	
Model 1	3.15 (2.77-3.58)	<0.001	3.69 (2.96-4.61)	<0.001
Model 2	3.16 (2.77-3.61)	<0.001	3.80 (3.04-4.74)	<0.001
Model 3	2.39 (2.05-2.79)	<0.001	2.59 (2.03-3.30)	<0.001
Model 4	1.55 (1.31-1.83)	<0.001	1.25 (0.95-1.63)	0.12
Model 5	1.64 (1.27-2.12)	<0.001	0.90 (0.66-1.24)	0.52
Normal AER to moderate albuminuria				
Individuals	*n=2,686*		*n=2,254*	
Events	*n=266*		*n=200*	
Model 1	2.42 (1.93-3.04)	<0.001	1.74 (1.19-2.57)	0.005
Model 2	2.40 (1.89-3.04)	<0.001	1.79 (1.21-2.64)	0.004
Model 3	1.65 (1.26-2.15)	<0.001	1.15 (0.77-1.73)	0.50
Model 4	1.64 (1.26-2.15)	<0.001	1.12 (0.74-1.69)	0.59
Model 5	1.59 (1.08-2.36)	0.02	0.82 (0.51-1.33)	0.41
Moderate to severe albuminuria				
Individuals	*n=544*		*n=405*	
Events	*n=110*		*n=86*	
Model 1	3.17 (2.29-4.38)	<0.001	3.32 (1.98-5.56)	<0.001
Model 2	3.06 (2.19-4.28)	<0.001	3.59 (2.09-6.16)	<0.001
Model 3	2.81 (1.91-4.11)	<0.001	3.37 (1.88-6.07)	<0.001
Model 4	2.79 (1.90-4.09)	<0.001	3.31 (1.84-5.95)	<0.001
Model 5	2.41 (1.44-4.06)	<0.001	1.81 (0.89-3.68)	0.10
Severe albuminuria to kidney failure				
Individuals	*n=649*		*n=426*	
Events	*n=266*		*n=165*	
Model 1	2.16 (1.75-2.66)	<0.001	3.60 (2.54-5.12)	<0.001
Model 2	2.19 (1.77-2.72)	<0.001	3.62 (2.54-5.14)	<0.001
Model 3	2.18 (1.72-2.77)	<0.001	3.41 (2.30-5.05)	<0.001
Model 4	1.24 (0.95-1.62)	0.12	1.03 (0.63-1.70)	0.90
Model 5	1.00 (0.63-1.60)	0.99	1.03 (0.57-1.86)	0.92

Data are hazard ratios with 95% confidence intervals for log-transformed remnant cholesterol (mmol/L; left column) or log-transformed apoC-III (mg/dL; right column).
Model 1 = unadjusted
Model 2 = diabetes duration + sex
Model 3 = Model 2 + HbA_{1c} + systolic blood pressure + smoking status + BMI
Model 4 = Model 3 + eGFR
Model 5 = Model 4 + apoC-III/remnant cholesterol

6.3.2 Remnant cholesterol variability

Consistent with the findings on remnant cholesterol concentration, we also noted higher remnant cholesterol variability with advancing baseline DKD stage: The median (IQR) of the CV was 23.1% (17.0-31.6%) in those with normal AER, 24.2% (18.1-32.7%) in those with moderate albuminuria, and 25.6% (19.5-34.8%) in those with severe albuminuria, p<0.001. The variability was higher among the DKD progressors than the non-progressors (27.3% [19.4-37.4%] vs. 23.2% [17.3-31.5%], p<0.001) with all study participants combined. A difference in the remnant cholesterol variability was only seen at the progression from normal AER to moderate albuminuria when separately evaluating the different steps of DKD progression, while no difference occurred at the later stages (Table 8).

Table 8 Variability of remnant cholesterol stratified by the diabetic kidney disease progression status during follow-up.

	Individuals	*Events*	No progression	Progression	p-value
All events pooled	*n=2,768*	*n=355*	23.2 (17.3-31.5)	27.3 (19.4-37.4)	<0.001
Normal AER to moderate albuminuria	*n=1,951*	*n=150*	22.7 (16.8-31.1)	27.8 (20.5-37.0)	<0.001
Moderate to severe albuminuria	*n=400*	*n=71*	23.7 (18.1-31.8)	30.0 (18.1-40.3)	0.07
Severe albuminuria to kidney failure	*n=417*	*n=134*	25.6 (19.6-34.1)	25.6 (19.1-36.1)	0.71

Variability (CV, coefficient of variation) is expressed as percentages and presented as median (interquartile range). Adapted from Study II (Table 4) with permission from the copyright holder.

We performed Cox regression analyses with similar adjustment patterns as were utilized for remnant cholesterol concentration to evaluate the association between variability and DKD progression further. The variability of remnant cholesterol in these analyses predicted the development of moderate albuminuria independent of sex and diabetes duration (HR 1.88 [95%CI 1.31-3.00], p<0.001), but the association was lost after further adjustment for modifiable covariates (HR 1.41 [95% CI 0.97-2.07], p=0.08). The HRs for any progression were 1.51 (1.20-1.90), p<0.001 (non-modifiable covariates) and 1.17 (0.92-1.49), p=0.19 (non-modifiable and modifiable covariates), respectively, with all study participants combined.

6.4 Diabetic kidney disease, cardiovascular disease, mortality, and the triglyceride-rich lipoprotein metabolism (Studies III-IV)

6.4.1 Remnant cholesterol, apoC-III, and cardiovascular events

The median follow-up time was 16.4 years (IQR 11.1-18.5; 66,055 person-years in total) in the remnant cholesterol analyses and 14.3 years (10.8-17.2; 48,078 person-years) in the apoC-III analyses with respect to the CVD outcome.

Remnant cholesterol concentrations were higher among the FinnDiane participants who suffered a CVD event during the prospective phase (0.51 mmol/L [IQR 0.38-0.74]) than those who did not (0.42 [0.33-0.57]), p<0.001. Using a Cox regression analysis (Table 9), we found a robust independent association between remnant cholesterol concentration and CVD events: The HR for log-transformed remnant cholesterol was 1.52 (95% CI 1.29-1.78), p<0.001, after adjustment for diabetes duration, sex, HbA_{1c}, systolic blood pressure, smoking status, BMI, eGFR, and LDL-cholesterol. LDL-cholesterol was included on top of the previously used model due to the close relationship between LDL-cholesterol and atherosclerotic CVD events (previously unpublished data, Jansson Sigfrids *et al.*).

The baseline concentration of apoC-III was similarly higher in those who suffered a CVD event (7.7 mg/dL [IQR 5.8-10.5] *vs.* 6.7 [5.4-8.7], p<0.001). The HR for apoC-III with respect to CVD events was 1.36 (95% CI 1.08-1.71), p=0.01, with the previously presented Cox regression adjustment model (Table 9). However, this connection lost significance (HR 1.04 [0.80-1.35], p=0.78) when remnant cholesterol was included in the model, in contrast to the association between remnant cholesterol and the outcome (HR 1.61 [1.29-2.02], p<0.001).

6.4.2 The impact of diabetic kidney disease (cardiovascular disease)

Considering the attained association between remnant cholesterol/apoC-III and DKD stage, as well as the well-recognized connection between DKD and CVD events, we stratified the cohort by DKD and assessed each stratum separately to account for the potentially confounding effect of kidney disease. This analysis used a three-staged classification of DKD in the interest of study power: normal AER, albuminuria (moderate to severe), and kidney failure. Cox models with different sets of covariates were applied.

Some features were noted regarding remnant cholesterol (previously unpublished data, Jansson Sigfrids *et al.*) from the acquired HRs that appear in Table 10. First, at the stage of normal AER and albuminuria, the relationship between remnant cholesterol and the CVD outcome was robust and independent of HbA_{1c}, systolic blood pressure, and eGFR, among other

potential confounders. The association was also, intriguingly, independent of LDL-cholesterol concentration, supporting the parallel atherogenicity of these lipoprotein particles. With Cox Model 4, the HR for LDL-cholesterol with respect to CVD was 1.25 (1.08-1.45), p=0.004 at the stage of normal AER and 1.12 (0.99-1.27), p=0.08 at the stage of albuminuria.

However, the second feature noted in the analyses was the lack of association between remnant cholesterol and CVD events among the study participants with kidney failure. This applied to all Cox models that were utilized. This also applied when stratifying the population into those on dialysis treatment (*n*=117) and those with a kidney transplant (n=211); p=0.92 and p=0.48 in unadjusted analyses, respectively.

Shifting the attention to apoC-III, the finding was as follows: The association between apoC-III concentration and CVD events was limited to the study participants with albuminuria, and among those, it was independent of the included nonmodifiable and modifiable confounders but not of eGFR and remnant cholesterol. Neither apoC-III nor remnant cholesterol concentration remained significantly associated with the outcome in the albuminuria subgroup with Model 5. The HR for log-transformed remnant cholesterol was 1.49 (1.07-2.05), p=0.02, and 1.38 (0.94-2.02), p=0.10, for log-transformed apoC-III when eGFR was removed from the model.

6.4.3 Remnant cholesterol, apoC-III, and mortality

Remnant cholesterol concentrations were also higher among the FinnDiane Study participants who died during the follow-up (0.55 mmol/L [IQR 0.40-0.79]) than those who did not (0.42 [0.33-0.57]), p<0.001. Furthermore, the same applied to the baseline apoC-III concentrations: 8.4 mg/dL (6.3-11.4) *vs.* 6.7 mg/dL (5.4-8.7), p<0.001.

The study participants were followed with respect to mortality for a median time of 16.3 years (IQR 10.7-18.5; 70,797 person-years in total) in the Study II cohort (remnant cholesterol) and 14.8 years (11.1-17.2; 53,319 person-years) in the Study III cohort (apoC-III).

The HR for log-transformed remnant cholesterol (Table 9) with respect to all-cause mortality was 1.47 (95% CI 1.26-1.73), p<0.001, after adjustment for diabetes duration, sex, HbA_{1c}, systolic blood pressure, smoking status, BMI, LDL-cholesterol, and eGFR. The HR for log-transformed apoC-III was 1.51 (1.21-1.88), p<0.001, with the same level of adjustment. The association between remnant cholesterol and mortality remained significant (1.45 [1.17-1.80], p<0.001) when both variables were included in the same Cox regression model, while the association between apoC-III and the outcome did not (1.24 [0.97-1.58], p=0.09).

Table 9 The association between remnant cholesterol/apoC-III and cardiovascular disease events/mortality: results from multivariable Cox proportional hazards regression analyses.

	Remnant cholesterol		ApoC-III	
	HR (95% CI)	p-value	HR (95% CI)	p-value
CVD events				
Individuals	*n=4,781*		*n=3,597*	
Events	*n=814*		*n=583*	
Model 1	2.04 (1.80-2.31)	<0.001		<0.001
Model 2	2.23 (1.96-2.54)	<0.001	2.49 (2.05-3.03)	<0.001
Model 3	1.81 (1.55-2.11)	<0.001	1.92 (1.54-2.38)	<0.001
Model 4	1.52 (1.29-1.78)	<0.001	1.36 (1.08-1.71)	0.01
Model 5	1.61 (1.29-2.02)	<0.001	1.04 (0.80-1.35)	0.78
Mortality				
Individuals	*n=5,150*		*n=3,966*	
Events	*n=904*		*n=653*	
Model 1	2.43 (2.16-2.72)	<0.001	3.85 (3.22-4.59)	<0.001
Model 2	2.60 (2.30-2.93)	<0.001	3.66 (3.07-4.38)	<0.001
Model 3	2.15 (1.86-2.49)	<0.001	2.69 (2.20.3.28)	<0.001
Model 4	1.47 (1.26-1.73)	<0.001	1.51 (1.21-1.88)	<0.001
Model 5	1.45 (1.17-1.80)	<0.001	1.24 (0.97-1.58)	0.09

Data are hazard ratios with 95% confidence intervals for log-transformed remnant cholesterol (mmol/L; left column) or log-transformed apoC-III (mg/dL; right column).
Model 1 = unadjusted
Model 2 = diabetes duration + sex
Model 3 = Model 2 + HbA_{1c} + systolic blood pressure + smoking status + BMI + LDL-cholesterol
Model 4 = Model 3 + eGFR
Model 5 = Model 4 + apoC-III/remnant cholesterol

6.4.4 The impact of diabetic kidney disease (mortality)

The findings for all-cause mortality followed the ones obtained for CVD when the cohort was divided into three strata of DKD (Table 10). Remnant cholesterol remained associated with the mortality outcome in the study population with normal AER until the step when eGFR was added as a covariate, which reduced the point estimate to 1.36 (0.91-2.01), p=0.07. ApoC-III was not associated with the mortality outcome in the unadjusted model or any of the models with eGFR in the normal AER group. Neither remnant cholesterol (HR 1.45 [0.96-2.20], p=0.08) nor apoC-III (HR 1.38 [0.84-2.28], p=0.20) remained significantly associated with all-cause mortality in this sub-population with a model including all covariates but except for eGFR.

Remnant cholesterol remained associated with the mortality outcome independently of all included covariates in the albuminuria group. ApoC-III, however, remained associated with the outcome independently of all covariates except for remnant cholesterol.

Finally, no association between remnant cholesterol/apoC-III and mortality was seen at any of the stages of adjustment in the kidney failure cohort.

Table 10 The association between remnant cholesterol/apoC-III and cardiovascular disease events/mortality: results from multivariable Cox proportional hazards regression analyses with stratification for diabetic kidney disease stage *(next page)*.

Data are hazard ratios with 95% confidence intervals for log-transformed remnant cholesterol (mmol/L) and log-transformed apoC-III (mg/dL). CVD events
to the left; all-cause mortality to the right.
Model 1 = unadjusted
Model 2 = diabetes duration + sex
Model 3 = Model 2 + HbA_{1c} + systolic blood pressure + smoking status + BMI + LDL-cholesterol
Model 4 = Model 3 + eGFR
Model 5 = Model 4 + apoC-III/remnant cholesterol

	CVD events				Mortality			
	Remnant cholesterol		ApoC-III		Remnant cholesterol		ApoC-III	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Normal AER								
Individuals	*n=3,070*		*n=2,496*		*n=3,173*		*n=2,603*	
Events	*n=277*		*n=223*		*n=208*		*n=177*	
Model 1	1.42 (1.10-1.83)	0.007	1.11 (0.76-1.63)	0.59	1.26 (0.93-1.71)	0.13	1.31 (0.86-2.02)	0.21
Model 2	1.89 (1.46-2.46)	<0.001	1.43 (0.96-2.12)	0.08	1.60 (1.170-2.20)	0.003	1.82 (1.16-2.84)	0.009
Model 3	1.71 (1.24-2.36)	0.001	1.25 (0.83-1.89)	0.29	1.56 (1.06-2.29)	0.02	1.67 (1.05-2.64)	0.03
Model 4	1.67 (1.21-2.31)	0.002	1.18 (0.78-1.80)	0.44	1.36 (0.91-2.01)	0.07	1.45 (0.91-2.32)	0.12
Model 5	1.86 (1.28-2.71)	0.001	0.88 (0.56-1.37)	0.56	1.34 (0.88-2.04)	0.17	1.26 (0.76-2.10)	0.37
Albuminuria								
Individuals	*n=1,193*		*n=878*		*n=1,327*		*n=1,008*	
Events	*n=373*		*n=260*		*n=395*		*n=284*	
Model 1	1.52 (1.27-1.83)	<0.001	1.80 (1.36-2.38)	<0.001	1.84 (1.55-2.18)	<0.001	2.46 (1.88-3.22)	<0.001
Model 2	1.77 (1.46-2.14)	<0.001	1.96 (1.47-2.60)	<0.001	2.12 (1.77-2.55)	<0.001	2.61 (1.99-3.42)	<0.001
Model 3	1.80 (1.39-2.34)	<0.001	1.80 (1.32-2.47)	0.002	2.18 (1.69-2.81)	<0.001	2.10 (1.55-2.82)	<0.001
Model 4	1.52 (1.15-2.01)	0.003	1.28 (0.91-1.79)	0.16	1.63 (1.25-2.14)	<0.001	1.44 (1.04-1.99)	0.03
Model 5	1.32 (0.95-1.85)	0.11	1.08 (0.73-1.60)	0.69	1.42 (1.03-1.96)	0.03	1.16 (0.79-1.69)	0.45
Kidney failure								
Individuals	*n=328*		*n=206*		*n=445*		*n=310*	
Events	*n=144*		*n=97*		*n=267*		*n=187*	
Model 1	1.08 (0.77-1.53)	0.64	1.11 (0.70-1.76)	0.66	1.16 (0.91-1.49)	0.23	1.20 (0.86-1.69)	0.28
Model 2	1.17 (0.82-1.66)	0.40	1.18 (0.74-1.88)	0.48	1.24 (0.96-1.60)	0.09	1.33 (0.95-1.87)	0.10
Model 3	1.05 (0.65-1.71)	0.83	0.92 (0.52-1.63)	0.79	1.15 (0.79-1.69)	0.47	1.38 (0.94-2.02)	0.10
Model 4	0.93 (0.56-1.53)	0.77	0.88 (0.49-1.58)	0.66	0.88 (0.59-1.32)	0.54	0.94 (0.62-1.41)	0.76
Model 5	1.40 (0.78-2.50)	0.26	0.75 (0.39-1.43)	0.38	1.17 (0.76-1.82)	0.48	0.89 (0.58-1.38)	0.60

6.5 Regression of diabetic kidney disease (Study IV)

6.5.1 Albuminuria regression rate

Of the 438 FinnDiane Study participants with a history of moderate albuminuria, 102 (23.3%) had regressed to a level of normal AER at baseline. Some 91 individuals had regressed to moderate albuminuria and 20 to normal AER of the 475 individuals with a history of severe albuminuria. These two groups were combined in the analyses; hence, the total severe albuminuria regression rate was 23.4%. A pictorial summary of albuminuria regression as a part of the natural history of DKD appears in Figure 24. The progression rates stem from Study I.

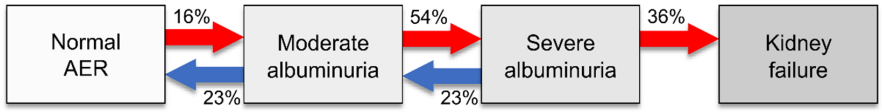


Figure 24 **The natural history of diabetic kidney disease: progression and regression rates.** The progression rates denote the 15-year progression risk and stem from Study I (1980-99 calendar-year diagnosis cohort); regression rates from Study IV.

6.5.2 Clinical characteristics associated with the regression of albuminuria

Table 11 summarizes the baseline clinical characteristics of individuals with prebaseline moderate and severe albuminuria stratified by regression status. A larger proportion of the regressors were women, and the regressors had both significantly lower blood pressure and better glycemic control than those whose albuminuria stage had sustained. The regressors were also characterized by a more favorable lipid panel regarding total cholesterol, LDL-cholesterol, triglycerides, and remnant cholesterol. However, some nonmodifiable variables such as age and diabetes duration did not differ between the groups.

A logistic regression analysis (previously unpublished data, Jansson Sigfrids *et al.*) with regression of albuminuria as the response variable and all univariately significant variables from Table 11 as covariates (total cholesterol and triglycerides were removed due to collinearity with LDL-cholesterol and remnant cholesterol, respectively, as evaluated with the variance inflation factor) showed that three variables remained significantly associated with regression of albuminuria: sex, with an odds ratio of 1.75 (95% CI 1.09-2.80, p=0.02, men as reference); HbA_{1c} (mmol/L), with an odds ratio of 0.98 (0.96-0.99, p=0.002); and apoC-III, with an odds ratio of 0.87 (0.78-0.97, p=0.01).

It is noteworthy that no difference in the median eGFR was observed when individuals with a history of albuminuria were combined; however, there was a difference in eGFR between the individuals with regressed and sustained severe albuminuria (Figure 25).

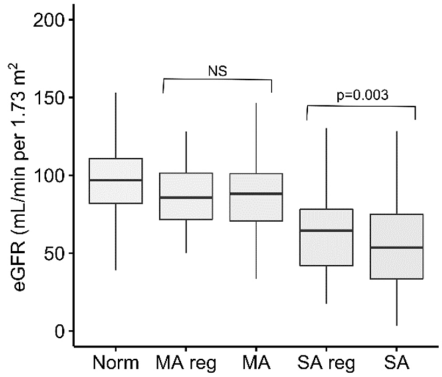


Figure 25 **Median, interquartile range (box), and range (whiskers) of eGFR (mL/min per 1.73 m^2) at the first FinnDiane visit**. Norm, normal AER; MA reg, moderate albuminuria regression; MA, sustained moderate albuminuria (no regression); SA reg, severe albuminuria regression; SA, sustained severe albuminuria (no regression). NS, non-significant.

Table 11 Clinical characteristics at the baseline FinnDiane visit stratified by albuminuria regression status.

	Regression	No regression	p-value
	n=213	*n=700*	
Age (years)	40.1±11.2	40.3±11.2	0.79
Age at diabetes onset (years)	10.9 (6.8-16.9)	10.6 (6.6-15.2)	0.61
Diabetes duration (years)	27.4±9.7	27.5±9.7	0.85
Sex (women)	107 (50%)	266 (38%)	0.002
HbA_{1c} (mmol/mol)	70.3±14.9	74.3±16.0	0.001
Systolic blood pressure (mmHg)	136±17	141±19	0.001
Diastolic blood pressure (mmHg)	79±10	82±10	<0.001
History of smoking			0.41
Current	199 (29%)	54 (26%)	
Former	190 (28%)	55 (26%)	
Never	287 (43%)	99 (48%)	
BMI (kg/m^2)	26.0±3.7	25.9±3.9	0.75
WHR, women	0.83±0.07	0.84±0.06	<0.001
WHR, men	0.92±0.06	0.93±0.07	<0.001
Total cholesterol (mmol/L)	4.93±0.91	5.28±1.03	<0.001
HDL-cholesterol (mmol/L)	1.24±0.36	1.26±0.40	0.57
LDL-cholesterol (mmol/L)	3.12±0.85	3.32±0.87	0.006
Triglycerides (mmol/L)	1.15 (0.81-1.61)	1.25 (0.92-1.82)	0.006
Remnant cholesterol (mmol/L)	0.48 (0.35-0.66)	0.51 (0.39-0.75)	0.009
ApoC-III (mg/dL)	6.5 (5.0-8.5)	7.5 (5.9-9.8)	0.002
eGFR (mL/min per 1.73 m^2)	76 (57-90)	72 (49-93)	0.13
Use of RAAS inhibitor	152 (71%)	524 (75%)	0.49
Use of lipid-lowering agent	139 (19.9%)	26 (12.2%)	0.01

Data are mean ± standard deviation, median (interquartile range), or n (%).

6.5.3 Regression of albuminuria and cardiovascular disease

Of the 3,449 study participants without a history of CVD events before entering the FinnDiane Study, 365 (10.6%) experienced a myocardial infarction, coronary procedure, or stroke during the follow-up phase in Study IV (median follow-up time 12.9 years (IQR 10.2-14.8); 41,023 person-years of follow-up in total). The Kaplan-Meier cumulative incidence curves in Figure 26 demonstrate that albuminuria and regression status at baseline had a major impact on the subsequent CVD event risk. The calculated 15-year cumulative event incidence rates were as follows: 8.0% (95% CI 6.7-9.3) for normal AER, 13.2% (6.1-19.7) for former moderate albuminuria (regression to normal AER), 21.5% (17.4-25.4) for moderate albuminuria (no regression), 28.7% (20.3-36.2) for former severe albuminuria (regression to moderate albuminuria or normal AER), and 40.1% (36.3-43.6) for severe albuminuria (no regression).

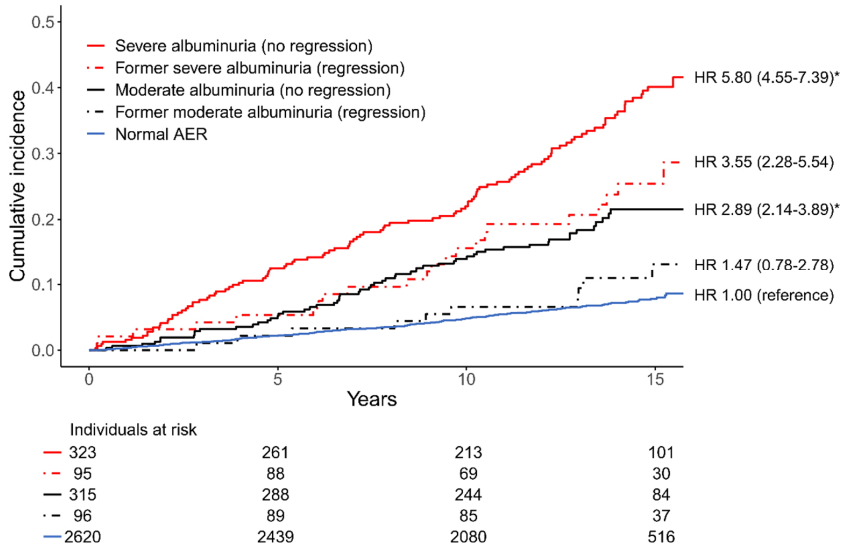


Figure 26 **Kaplan-Meier incidence curves for cardiovascular disease**. The groups are stratified by albuminuria status at the first FinnDiane visit. * denotes p<0.05 *vs.* preceding group. AER, albumin excretion rate; HR, hazard ratio. Copyright © 2018 Springer Nature. Adapted from Study IV (Figure 2) with permission from the copyright holder.

The CVD risks per albuminuria groups are also presented as HRs in Figure 26 (derived from unadjusted Cox regression analyses). The Cox regression analyses revealed, interestingly, that the CVD risk for the former moderate albuminuria (regression) group was no different than that of the normal AER group (p=0.24), whereas it was significantly lower compared to the moderate albuminuria (no regression) group (o=0.049). The same phenomenon was

noted for the study participants with a history of severe albuminuria: If regression to moderate albuminuria had occurred, the CVD risk was comparable to that of the moderate albuminuria (no regression) group (p=0.43) but significantly lower than what was observed for the severe albuminuria (no regression) group (p=0.03).

We performed Cox regression analyses with different levels of adjustment with albuminuria regression (yes *vs.* no) as an explanatory variable to further investigate the association between albuminuria regression and the CVD risk. These analyses combined the individuals with histories of moderate and severe albuminuria (n=829 in total). Table 12 presents the results. Regression of albuminuria was associated with a subsequently diminished CVD risk in the unadjusted analysis, and adjustment with the non-modifiable covariates age, age at diabetes onset, and sex did not affect the level of association (HR 0.57 [95% CI 0.39-0.83], p=0.003). However, the association did not persist after further adjustment with modifiable covariates (HbA_{1c}, systolic blood pressure, WHR, remnant cholesterol, and eGFR). Of note, despite the sex distribution within the regression and non-regression groups differing, the relationship between albuminuria regression and CVD was not modified by sex (p for interaction=0.26).

Table 12 The impact of regression of albuminuria on the risk of cardiovascular events and all-cause mortality: results from a Cox proportional hazards regression analysis.

	CVD		Mortality	
Individuals	*n=829*		*n=913*	
Events	*n=199*		*n=233*	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Model 1	0.57 (0.39-0.82)	0.004	0.55 (0.38-0.79)	0.001
Model 2	0.57 (0.39-0.83)	0.003	0.54 (0.38-0.78)	<0.001
Model 3	0.69 (0.47-1.02)	0.06	0.59 (0.40-0.86)	0.006
Model 4	0.76 (0.51-1.13)	0.17	0.64 (0.44-0.94)	0.02

Data are hazard ratios with 95% confidence intervals for regression (yes *vs.* no).
Model 1 = unadjusted
Model 2 = age + age at diabetes onset + sex
Model 3 = Model 2 + HbA_{1c} + systolic blood pressure + WHR + remnant cholesterol
Model 4 = Model 3 + eGFR

6.5.4 Regression of albuminuria and mortality

The study cohort was followed for the vital status over a median follow-up time of 14.0 years (IQR 11.6-15.9) and for 47,513 person-years overall. During the observation period, 370 study participants (10.2% of the cohort) died. The cause of death – either underlying, immediate, or both – was a disease of the circulatory system in 53.8% of the cases.

Albuminuria status at baseline influenced the succeeding death rate in the same manner as was previously reported for CVD events. In other words, the mortality risk was significantly higher in the severe albuminuria (no regression) group than the former severe albuminuria (regression) group (p=0.02), but no difference between the latter and the moderate albuminuria (no regression) group (p=0.14) was seen. The risk was also significantly higher among those with moderate albuminuria (no regression) than those with former moderate albuminuria (regression; p=0.03), but not different between those with former moderate albuminuria (regression) and the normal AER group (p=0.34). The phenomenon is illustrated by the Kaplan-Meier survival curves in Figure 27. The calculated 15-year mortality rates were 6.1% (95% CI 5.2-7.0) for the normal AER group, 8.8% (3.3-14.0) for the former moderate albuminuria group, 19.2% (15.5-22.8) for the moderate albuminuria, 25.1% (18.5-31.2) for the former severe albuminuria group, and 38.1% (34.9-41.2) for the severe albuminuria group. No interaction between sex and albuminuria was observed regarding all-cause mortality.

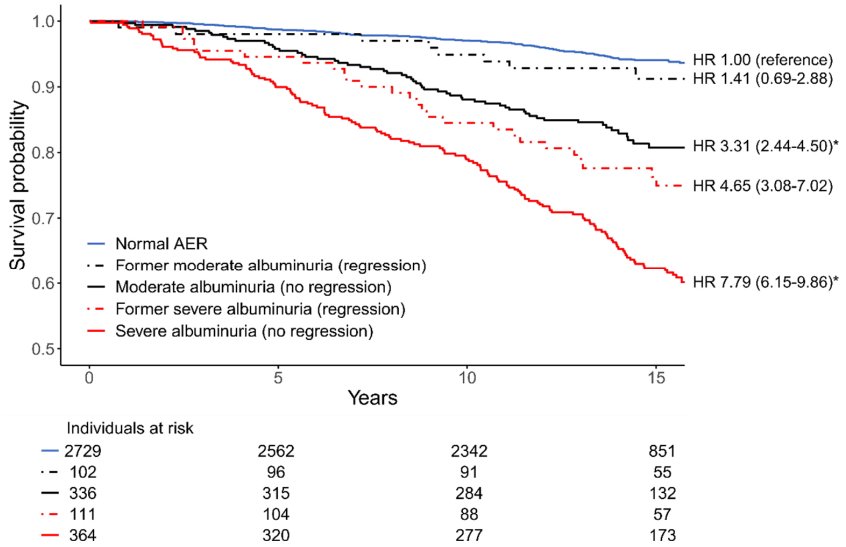


Figure 27 **Kaplan-Meier survival curves (all-cause mortality)**. The groups are stratified by albuminuria status at the first FinnDiane visit. * denotes p<0.05 *vs.* preceding group. AER, albumin excretion rate; HR, hazard ratio. Copyright © 2018 Springer Nature. Adapted from Study IV (Figure 3) with permission from the copyright holder.

Table 12 shows the results from Cox regression analyses with respect to all-cause mortality in the second panel. The regression of albuminuria remained significantly associated with mortality, independent of the most stringent adjustment model (HR 0.64 [95% CI 0.44-0.94], p=0.02).

7 DISCUSSION

There has been a substantial gap in the knowledge of the contemporary natural history of DKD in type 1 diabetes, particularly regarding the epidemiology of albuminuria. No reports designated explicitly to this topic have previously been published from Finland, and the data from other countries are also limited. Thus, the cumulative incidences, incidence rates patterns, and temporal trends we show in Study I are novel. Study IV is also the first of its kind, to the best of our knowledge, to show an association between regression of albuminuria and a reduced burden of CVD events and premature mortality in type 1 diabetes. Furthermore, this thesis introduces remnant cholesterol and apoC-III – both key players in the TRL metabolism – into the context of DKD in type 1 diabetes. We show that in type 1 diabetes, the association between apoC-III and macrovascular endpoints is limited to the individuals with albuminuria. However, the results of this thesis need to be interpreted with attention to its strengths and limitations.

7.1 Strengths and limitations

Study populations One of the main strengths of this thesis is the cohort of Study I, which is population-based, stems from random sampling, and is therefore representative of the Finnish populace with type 1 diabetes. The sampling size was derived from power calculation and is large in relation to what has been published in the past within the topic. Another strength is that we followed the study subjects from their diabetes diagnoses onwards and the obtained follow-up times are long in comparison to previous work (up to 50 years of diabetes duration per participant was covered). All Finnish residents are offered medical care services in the public health care system; thus, most individuals with type 1 diabetes attend diabetes outpatient clinics at the public hospitals or primary health care centers, which enabled the acquisition of medical records for 95% of the original cohort. However, a feature of the study design that could be viewed as a limitation is that only a few clinical characteristics (the year of diabetes duration, age at diabetes duration, and sex) were available as potential confounders in the multivariable analyses. Thus, we were unable to investigate the impact of, for instance, glycemic control and lipids on the natural history of DKD. However, these were evaluated in detail in Studies II-IV in the FinnDiane cohort.

The FinnDiane Study is the largest well-characterized type 1 diabetes research cohort with and without DKD in the world. Participants have been recruited for 25 years, and many are recurrently re-examined at the FinnDiane centers. The same standard examination protocol is utilized for every individual at every visit. The possibility of data linkage between clinical-visit

data and register-derived data through personal identity codes is also unique. However, despite representing ~10% of the individuals with type 1 diabetes in Finland evenly distributed around the country, the study is not population-based by strict definitions. The cohort might be afflicted by self-selection bias by recruiting adult volunteers, because the health behavior of the recruited participants may differ from what would be observed in a random sample – a phenomenon that has been previously noted in observational, prospective health studies [301,302].

Classification of diabetes Many factors point towards an accurately classified diabetes type within the cohort, even though the measurement of diabetes-related autoantibodies is not included in the FinnDiane Study protocol. First, only individuals with type 1 diabetes according to the conventional diagnostic criteria are recruited to the FinnDiane Study. According to the national and international guidelines for unraveling the type of diabetes, the autoantibodies have been checked for most – if not all – individuals when their diabetes diagnoses were set. Second, as a "quality check" to omit any potential study participants with an incorrect diagnosis of type 1 diabetes, age at diabetes onset under the age of 40 and transition to insulin treatment within one year were set as inclusion criteria in Studies II-IV. The classification of diabetes type in those with diabetes onset between 30 and 40 years could be considered arbitrary. However, a subpopulation of the FinnDiane Study participants with diabetes onset at >35 years has been genotyped for genes typically associated with type 1 diabetes (HLADQB1, PTPN22, INS, and CTLA4). A significant genetic difference was observed between these individuals and individuals with established latent autoimmune diabetes in adults (LADA), and the prevalence of risk genotypes was also similar to the study participants with diabetes onset before the age of 20 years [303]. In other words, this further supports that the diabetes type classification in the FinnDiane Study is precise.

The medical records of five individuals in Study I revealed clinical characteristics that are not typical to type 1 diabetes; thus, these individuals were excluded. No concerns of this type were raised for the remaining study participants. The original cohort in Study I is compiled from several overlapping sources, so it is unlikely that any cases of type 1 diabetes are missing.

Definition and ascertainment of endpoints In all studies included in this thesis, the classification of albuminuria was based on international reference limits with careful scrutiny for potential distorting circumstances, such as fever, menstruation, pregnancy, or urinary tract infections. Two positive samples out of three consecutive ones were required to avoid diagnosing transient spikes in AER as persistent albuminuria. The progression status was retrieved from medical records instead of relying on self-reported questionnaires, which reduced the likelihood of recall bias and is a key strength of this thesis.

However, a few potential limitations regarding the characterization of DKD should be noted.

First, we cannot entirely rule out the risk that the different albuminuria assessment methods covered, and the changes in their representation over time have influenced the results. However, it is important to bear in mind that the albuminuria assessment methods incorporated in this thesis represent the development of the albuminuria testing according to the globally recommended and used clinical guidelines. Furthermore, as pointed out before, a high agreement between early morning ACR and 24-hour AER regarding the categorization of albuminuria has been established in type 2 diabetes [60] and is therefore also surmised in type 1 diabetes.

Second, despite the fact that Study I is a factual representation of the incidence rates of moderate and severe albuminuria, it is noteworthy that the study solely investigated albuminuria progression, not regression. Thus, the results represent incident albuminuria cases but not necessarily persistent ones.

Another potential limitation is the definition of regression in Study IV. We looked at regression cross-sectionally at the first FinnDiane visits and might therefore have missed some fluctuations in the AER levels, as will be discussed in detail below. Furthermore, we did not have the possibility to examine changes in continuous AER/ACR measurements but instead assessed regression as a categorical trait (change to a less advanced category of albuminuria; yes or no). By this, we could theoretically have lost some information about diminished AER levels. However, two extensive studies that examined albuminuria regression in the past, one in type 1 [90] and the other in type 2 diabetes [304], found regression rates strikingly alike when defining regression as a 50% reduction in AER (regression rates 58% and 51%, respectively) *vs.* as a categorical change (reversion from moderate to normal AER; yes or no; 59% and 54%, respectively). Therefore, it is unlikely that our choice of outcome classification produced considerably different results than what would have been obtained if continuous AER determinations had been available for all individuals.

The register-derived endpoints in this thesis descend from well-established and validated nationwide registers. The Care Register for Health Care, from which the CVD events were obtained, has a positive predictive value of 75 to 99% for common diagnoses, including myocardial infarctions and strokes [305]. Moreover, the Finnish Cause of Death Register (from which the vital statuses were retrieved) relies on data from death certificates with verification via the Population Information System; thus, its coverage is nearly 100% [306].

Statistical analyses In the multivariable analyses of this thesis, the choice of confounders was critically deliberated, variable distributions assessed, and continuous confounders included in the models according to the observed variable distributions or known relationship with the outcomes of interest.

However, regarding the statistical analyses, it is necessary to recognize potential problems arising from the use of a single time-point measure *vs.* a time-dependent variable. The Cox model appraises the effect of covariates, assuming that their effects are the same at any point on the time scale. If it is likely that a covariate varies over time, thus giving rise to changes in the HRs, it does not fulfill the proportional hazards assumption and may result in estimates of incorrect magnitude. The available confounders in Study I were stable over time (diabetes diagnosis year, age at diabetes onset, and sex), so this type of concern was not raised. Only one apoC-III measurement per participant was available in Study III, and this is a limitation of that study. However, Study II also assessed a longitudinal metric – namely its visit-to-visit variability – in addition to the baseline-measured remnant cholesterol concentration. The correlation between the mean serial remnant cholesterol, which was used for the variability calculations, and the remnant cholesterol concentration measured at the first FinnDiane visit, was high (correlation coefficient=0.78, $p<0.001$), diminishing the likelihood of the bias outlined earlier. It is further of note that a high correlation between baseline HbA_{1c} (covariate in the Cox regression analyses in Studies II-IV) and mean serial HbA_{1c} has been earlier observed in the FinnDiane Study (correlation coefficient=0.72, $p<0.001$) [134].

Generalization of findings Besides the potential methodological issues that may affect the internal validity of the results, some demographic factors may limit the external validity and should be acknowledged. These factors are that 1) the Finnish population is characterized by genetic homogeneity, 2) most of the populace is White, and 3) Finland has a higher incidence of type 1 diabetes than any other country in the world [5].

7.2 Incidence of diabetic kidney disease

Study I assessed the incidence rates of the different stages of albuminuria for the first time in Finland with a population-based study design. We reported crude diabetes duration-specific incidence rates, incidence rate patterns, cumulative incidences, and their secular trends in a large cohort of individuals with the debut of type 1 diabetes in the 1970s, 1980s, and 1990s.

7.2.1 The calendar effect of albuminuria has reached a plateau

The cumulative incidence rates of DKD in the 1970-79 diagnosis cohort were comparable to the early reports: After 25 years of diabetes, 27% had developed severe albuminuria, and the proportion was 38% after 45 years. The analyses revealed that the outlook had improved with increasing calendar years of diabetes: The 25-year cumulative incidence of severe albuminuria was only

12% in the 1980-89 diagnosis cohort. However, a plateau phase had been reached after this; the 25-year cumulative incidence in the 1990s cohort was 11%. Of note, the results obtained for moderate albuminuria agreed with the above: 30% had developed moderate albuminuria 25 years after the onset of diabetes in the 1980-89 cohort and 31% in the 1990-99 cohort.

Figure 28 presents groundbreaking events that may have contributed to the improved prognosis of DKD. These include advances in glucose monitoring, AER determination, and blood pressure control. Despite the diabetes treatment regimens having taken giant leaps also during more recent years (new insulin agents, continuous glucose monitoring devices *etc.*), most of the presented inventions took place in the early 1980s. Indeed, this might explain, at least in part, the out-leveled calendar effect.

7.2.2 Are we preventing or delaying diabetic kidney disease?

The characteristic natural history of albuminuria with an incidence peak ~15 years after the onset of diabetes and a consequent rapid incidence decline has been thought to arise from a genetic predisposition to DKD; if renal complications developed entirely because of a cumulative burden of hyperglycemia, the incidence would continue its steady rise until most individuals would be affected [15]. The early studies on proteinuria projected that only 45-50% of people with type 1 diabetes would ever develop DKD because of this [14].

We replicated the existence of an incidence peak of severe albuminuria in the 1970-79 diagnosis cohort; with 5-year intervals of diabetes duration as strata, the highest incidence rate was seen at 15-19 years of diabetes. However, in the two later diagnosis cohorts (1980-99), we could not see a peak in the duration-specific incidence rate anymore. After an initial rise, the incidence promptly evened out to an average rate that was under half of the peak incidence rate in the 1970-79 diagnosis cohort. Thus, it seems that the groundbreaking inventions and interventions presented in Figure 28 might be postponing the onset of albuminuria in some individuals who are genetically predestined to develop DKD.

However, whether we are only delaying or also preventing some cases of DKD is a fundamental question. It is noteworthy that despite the substantially lower incidence rates in the 1980-99 than the 1970-79 cohort at a diabetes duration of 5 to 24 years, the situation reversed at years 25-29: then, the incidence rate was over two-fold in the 1980-99 cohort compared to the earlier one, which had already passed its incidence peak. The follow-up was limited to a maximum of 40 years in the 1980-99 cohort, but the number of individuals after 30 years, and especially after 35 years, was quite low. Therefore, a re-analysis should be done when the follow-up times can be prolonged to investigate at what level the incidence rate pattern eventually arrived. Given that the GAM modeling revealed a downward trend of the moderate

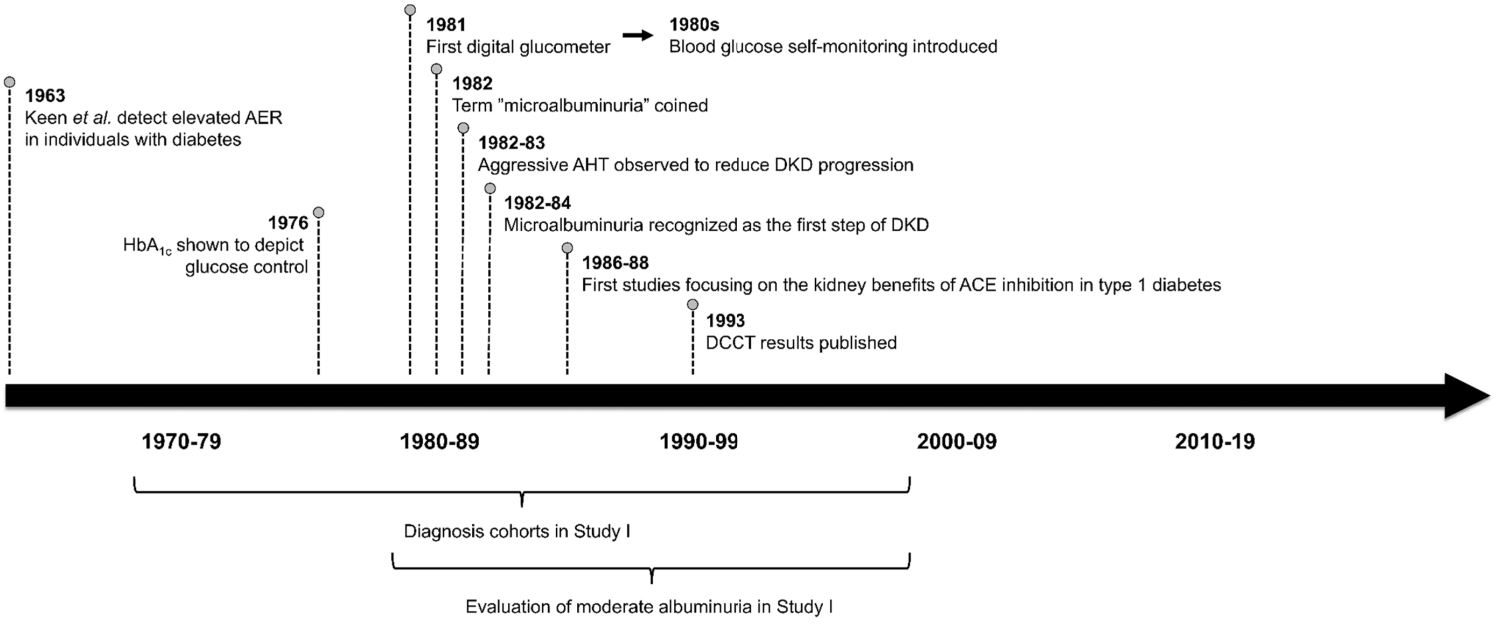

1963
Keen et al. detect elevated AER in individuals with diabetes
1976
HbA1c shown to depict glucose control
1981
First digital glucometer
1980s
Blood glucose self-monitoring introduced
1982
Term "microalbuminuria" coined
1982-83
Aggressive AHT observed to reduce DKD progression
1982-84
Microalbuminuria recognized as the first step of DKD
1986-88
First studies focusing on the kidney benefits of ACE inhibition in type 1 diabetes
1993
DCCT results published
1970-79
1980-89
1990-99
2000-09
2010-19
Diagnosis cohorts in Study I
Evaluation of moderate albuminuria in Study I

Figure 28 **Inventions and important discoveries that have likely contributed to the decreased cumulative incidence of diabetic kidney disease in type 1 diabetes** *(previous page)*. The included calendar-year diagnosis cohorts (1970-79, 1980-89, and 1990-99) are also pointed out, as well as those diagnosis cohorts in which moderate albuminuria (microalbuminuria) was assessed (1980-89 and 1990-99). References for the presented points: Keen *et al.* [307]; HbA_{1c} [308]; development of glucose monitoring [309]; "microalbuminuria" coined [17]; aggressive antihypertensive treatment (AHT) introduced to DKD [310,311]; microalbuminuria recognized as a step in DKD [17–19]; first studies focusing on kidney benefits of ACE inhibition in type 1 diabetes [312–314]; the groundbreaking findings of the Diabetes Control and Complications Trial (DCCT) [131].

albuminuria incidence rate from 25 years of diabetes onwards, it is possible that a decline in the severe albuminuria rate will also be attained before the cumulative incidence gap between the calendar-year cohorts has been closed.

7.2.3 Moderate albuminuria progresses frequently

The progression of moderate to severe albuminuria was only evaluated in the 1980-89 and 1990-99 diagnosis cohorts, because the availability of early albuminuria determinations was limited in those formerly diagnosed. Therefore, a comparison of the progression rate with the 1970-79 diagnosis cohort was not possible.

Five years after the appearance of moderate albuminuria, one-third of the patients had advanced to the following DKD stage, and this proportion had increased to approximately half when 15 years had passed. This is considerably lower than what was seen in the seminal studies 40 years ago [17–19], although considerable risk of progression from the first stage of DKD to the more advanced ones persists.

7.2.4 The progression rate to kidney failure has remained unchanged

The progression rate between severe albuminuria and kidney failure remained unchanged in contrast to the decreased cumulative incidence of severe albuminuria over time: 15 years after the first appearance of severe albuminuria, roughly 35% of the individuals at risk – both in the 1970-79 and the 1980-99 cohorts – had advanced to kidney failure.

The course between severe albuminuria and kidney failure was previously evaluated in the DCCT, and of note, the 15-year cumulative progression rate was only around 20% [93]. The reasons for this large discrepancy are unclear. However, one must keep in mind that the DCCT cohort represents a selected, frequently monitored, and meticulously guided group of individuals. In contrast, a nationwide, population-based cohort such as ours also includes the individuals who cannot adhere to self-care and the other treatment recommendations, and these persons would be very unlikely to participate in

studies like the DCCT. Indeed, depression [315], lack of social support [316], alcohol misuse [317], illicit drug use [318], and the fear of hypoglycemia [319] have been associated with poor glycemic control in diabetes. A meta-analysis showed that depression was also directly associated with an increased risk of diabetic micro- and macrovascular complications [320]. Therefore, it is possible that these factors impact the difference in progression rates. Consequently, they may also need more focus when making treatment plans for persons with severe albuminuria.

7.2.5 The sex difference in diabetic kidney disease risk is specific to the age at diabetes onset – are sex hormones implicated?

We observed that the cumulative risk for both moderate and severe albuminuria was the lowest if type 1 diabetes had occurred at a young age, that is, before the age of 5. The delay compared to the later onset groups was approximately 4-8 years. There was no difference in the risk between the 5-9- and 10-14-year groups. The same observation has been previously made by some others [75,115,116]. Thus, it has been postulated that the peri- and post-pubertal years of diabetes contribute to the DKD risk more than the prepubertal years do. People have tried to speculate about the reasons for this phenomenon, but it is still an open question. One possible explanation could be the peaking growth hormone concentrations during the pubertal maturation and the subsequent rise in lipolysis, glycogenolysis, gluconeogenesis, and other components of insulin resistance. Additionally, low concentrations of insulin-like growth factor 1 (IGF-1) as compared to non-diabetic counterparts are seen during puberty in individuals with type 1 diabetes, especially in those with poor diabetes control. The disproportionately low IGF-1-levels could, hypothetically, give rise to a negative feedback loop and by that lead to further increased growth hormone levels. Insulin resistance is known to be associated – possibly even causally – with DKD, which could explain why the prepubertal years of diabetes do not add to the cumulative DKD risk as much as the later years do [321]. Another plausible factor is that the adaptation to diabetes management may be more straightforward in individuals with early-onset disease than those with disease debut around puberty.

The impact of sex on the DKD risk has been an area of debate, with studies suggesting risk excess in men, women, or no risk difference between the sexes (Section 2.2.4). We found a male preponderance for moderate and severe albuminuria (Study I) that did not manifest before 20 years of diabetes and only if diabetes had been diagnosed at an age between 10 and 14 years. If that was the case, the DKD risk in men was 1.5-fold for moderate and 1.8-fold for severe albuminuria, whereas the risk did not differ between the sexes if diabetes had occurred earlier. This is not the first time such observations have been made, interestingly, because reports from the FinnDiane Study [121], the

Finnish Kidney Registry [100], and a Swedish cohort [103] have previously arrived at similar conclusions for kidney failure. The underlying explanation is unknown, but sex hormones have been implicated.

The putative role of sex hormones stems from the nondiabetic population. It is well known that women exhibit lower risks for vascular diseases, such as CVD [322] and CKD [323], than age-matched men without diabetes; however, the protection of the female sex is lost after menopause. Based on these findings, ovarian hormones have been considered protective of the vasculature. The understanding is further supported by experimental studies that have revealed attenuation of kidney damage after administration of 17β-estradiol to rodents, both female and male [324].

Diabetes, conversely, is associated with hormonal dysregulation, especially with diminished circulating estradiol concentrations in women [325,326]. Women with type 1 diabetes also experience menstrual cycle abnormalities, adverse pregnancy outcomes, early menopause, and delayed menarche more often than women without diabetes [326,327].

Taken together, the role of sex hormones in the DKD pathogenesis is possible. The aspect of diminished circulating estrogens in women with diabetes could explain why vascular protection in women is lost or at least weakened if diabetes develops. However, a large knowledge gap exists, because no studies exist of the connection between sex hormones and the development of diabetic complications in women.

Nevertheless, *how does this align with our finding that the sex difference in DKD is specific to age at diabetes onset?* We hypothesize that the supposedly renoprotective role of estrogen does not take effect unless the diabetes onset overlaps with or occurs later than the pubertal rise in estrogens. In other words, the timing and order of events, such as hormonal changes and the onset of diabetes, may play a role in determining the risk of microvascular comorbidities [121]. As for the effect translation, genetic and epigenetic mechanisms (*interaction between hyperglycemia and sex hormones?*) have been speculated upon but need considerably more investigation [120,121].

7.2.6 Survival after the onset of albuminuria

Many breakthroughs have occurred during the past decades regarding antihypertensive agents and their role in the treatment of DKD. The prognosis for the afflicted individuals was grim before their development when no pharmacological prevention or therapy for DKD was available: The median survival after onset of proteinuria was only 5-6 years [224,225]. Two papers in 1982 and 1983 showed beneficial effects of aggressive antihypertensive medication (the studied medications were diuretics, beta blockers, and/or alpha blockers) in DKD [310,311], and the first studies showing salutary renal effects of ACE inhibitors in type 1 diabetes were published some years later [312–314]. This, together with the other advances in diabetes care (Figure 28),

led to substantial improvement in the DKD prognosis: Astrup *et al.* showed that the median post-DKD prognosis had extended to over 20 years in a cohort with an average type 1 diabetes duration of nearly 30 years at the study baseline in 1993 (meaning that the study participants had been diagnosed with diabetes around the 1960s) [328]. However, the 10-year cumulative mortality rate was nearly 25% in the cohort after the onset of severe albuminuria, [328].

Our results from Study I are even more hopeful, because only 11% of the study participants with diabetes onset between 1970 and 1999 had died 10 years after the first appearance of severe albuminuria. However, our analysis of the causes of death after a diagnosis of severe albuminuria (46% died of cardiovascular disease, 34% of multiple complications of diabetes) highlights an important dilemma: *how to further reduce the vascular burden carried by individuals with albuminuria?* Based on strong and stepwise increasing association between renal complications and premature mortality, preventing the development and progression of DKD needs to be aimed at, and individuals at high risk need to be identified at an early stage for this purpose. Studies II and III show novel associations between key components of the TRL metabolism and kidney endpoints in a type 1 diabetes population, thus introducing these as potential risk factors.

7.3 Progression of diabetic kidney disease

Study II evaluated the associations between remnant cholesterol (calculated as the non-HDL, non-LDL cholesterol), DKD stages (cross-sectional analyses), and the progression of DKD (prospective analyses). Study III assessed the relationship between apoC-III concentration and DKD, also accounting for remnant cholesterol and other lipid- and non-lipid confounders.

7.3.1 Association with remnant cholesterol and apolipoprotein C-III

We noted increasing baseline concentrations of remnant cholesterol and apoC-III with an advancing stage of DKD in the cross-sectional analyses. The results further revealed that remnant cholesterol was a robust predictor of the progression of DKD. The association between remnant cholesterol and the DKD outcome was seen at all levels of progression independent of several other powerful covariates, except for eGFR at the stage of severe albuminuria to kidney failure. The HR for remnant cholesterol was 1.64 after adjustment for HbA_{1c}, systolic blood pressure, eGFR, and apoC-III, among other factors, when all progression events were pooled.

ApoC-III was strongly associated with the progression from moderate to severe albuminuria, albeit not independent of remnant cholesterol. At the first level of progression – from normal AER to moderate albuminuria – glycemic control (as HbA_{1c}) appeared to mediate most of the effect, whereas at the third

level of progression – from severe albuminuria to kidney failure – kidney function (as eGFR) appeared to be the primary effect mediator.

7.3.2 What links lipids with diabetic kidney disease?

Experimental studies have provided a biological basis for the connection between remnant cholesterol and atherosclerosis [195]. However, despite the fact that diabetic micro- and macrovascular complications tend to coincide, the relationship between remnant cholesterol and DKD is not as apparent, because atherosclerosis is not considered the driving mechanism of DKD. It is, of course, possible that remnant cholesterol serves as a proxy for another variable that was not accounted for in this thesis. Moreover, as with all observational studies, directionality is key: *does dyslipidemia drive DKD or DKD dyslipidemia*? Nevertheless, some possible mechanisms exist that could connect remnant cholesterol with kidney disease.

The first piece of evidence derives from animal studies: Glomerulosclerosis, increased glomerular pressure, and tubulointerstitial damage have been observed after diet-induced hypercholesterolemia in rodent models [329]. Studies on Nagase analbuminemic rats by Joles *et al.* have been particularly informative. The female Nagase analbuminemic rats are severely dyslipidemic and are predominantly characterized by high cholesterol- and triglyceride content in VLDL and IDL particles. The female rats, intriguingly, develop spontaneous proteinuria, and significant glomerular lipid deposition, and glomerulosclerosis is apparent in renal histological analyses [330]. The male Nagase analbuminemic rats are less dyslipidemic and have lower levels of VLDL and IDL lipoproteins. The male models only develop mild proteinuria and remain free of the distinctive histological kidney pathologies [330].

A second potential explanation is the monocyte-foam cell-inflammation-axis. High VLDL enhances the expression of monocyte chemoattractant protein 1 in mesangial cells in experimental models, increasing the adhesion of monocytes to the mesangium [331]. The monocytes differentiate into macrophages and through lipid-ingestion to foam cells, which secrete proinflammatory mediators – thus creating a ”positive feedback loop” that augments itself [332]. Varbo *et al.* have reported an association in line with this hypothesis between remnant cholesterol and low-grade systemic inflammation in man [333].

A third factor, hypercholesterolemia – like obesity, insulin resistance, and diabetes – has been associated with increased endothelin production in the kidney. Endothelins, especially endothelin-1 acting through the endothelin-A receptor isoform, have been implicated in diabetic and non-diabetic CKD. Binding of the receptor isoform leads to local vasoconstriction, mesangial proliferation, systemic hypertension, and numerous other effects that are not only critical in maintaining renal perfusion but are also key in the progression of kidney disease. Several clinical trials of endothelin antagonists in CKD are

ongoing or have recently been completed, so the understanding of lipids, endothelins, and kidney disease will probably expand during the following years [334].

Taken together, some evidence exists for a role of (TRL) lipids in DKD, although the knowledge is still limited. In this context, *what is the role of apoC-III*? A very recent study of diabetic mice showed that apoC-III overexpression activated the renal Toll-like receptor 2 and nuclear factor-κB signaling pathways and increased the renal expression levels of downstream inflammatory factors. Aggravation of early kidney injury was, intriguingly, noticed in association with this [335]. Additionally, because the actions of apoC-III impair the clearance of TRL remnants, one is, indeed, tempted to speculate that apoC-III is a mediating factor or even an initiator of the sequence that leads to increased remnant cholesterol and results in kidney injury. The fact that adjustment for remnant cholesterol reduced the risk estimate of apoC-III in Study III could be seen to reinforce this theory. Section 7.4 discusses this hypothesis, but in relation to CVD and mortality, in detail.

7.3.3 High remnant cholesterol variability does not predict the progression of diabetic kidney disease

An interest in the association between intraindividual variability of clinical variables and outcomes such as CVD events and kidney disease has appeared during recent years. The relation between HbA_{1c} variability and diabetic complications has been in focus in the context of type 1 diabetes, whereas lipid variability has received less attention. Research involving individuals with type 2 diabetes has proposed that high variability of triglycerides and HDL-cholesterol predict the progression of kidney disease [147,148]. We observed a median remnant cholesterol variability of 23 to 30% in Study II that was dependent on the DKD progression status. However, our results from multivariable analyses do not support an association between remnant cholesterol variability and kidney outcomes in type 1 diabetes.

7.4 Diabetic kidney disease, cardiovascular disease, mortality, and the triglyceride-rich lipoprotein metabolism

The question of confounding is key when interpreting our results on remnant cholesterol, apoC-III, CVD events, and mortality. The next section covers confounding as a phenomenon in general, and after that, in relation to Studies II and III.

7.4.1 Confounding is a threat to the interpretation of observational studies

Observational studies do not have the potential to reveal causality; therefore, randomized-controlled trials are the gold standard in the process of uncovering causal interference in clinical research. Nevertheless, observational studies play an important role in hypothesis generation, and observational study designs are excellent initial tools when aiming to explore the relationship between an exposure variable and an outcome [336]. However, the potential flaws of confounding should always be acknowledged when assessing the internal validity of observational studies, because confounding is a significant threat to the evaluation of exposure-outcome relationships.

A confounding variable can be viewed as an alternative explanation for the outcome of interest. A variable needs to fulfill three criteria to be a confounder: 1) it is associated with the exposure variable; 2) it is associated with the outcome variable; and 3) it is not directly affected by the exposure and is not an intermediate variable in the causal pathway between the exposure and the outcome of interest [337]. There are some ways to control for confounding, which include performing a randomized controlled trial instead of an observational study (however, that is not always possible, *e.g.*, due to ethical considerations); matching (*i.e.*, cases and controls are matched, *e.g.*, by DKD status); and stratification (*e.g.*, to study individuals with and without DKD separately, or to only recruit individuals without DKD in the first place, then referred to as restriction) [337]. Furthermore, the use of multivariable regression models – as was done in this thesis – enables controlling for several confounders at the same time. However, multivariable modelling also comes with some limitations that must be recognized. Adjustment is only possible for known and available confounders, and inaccurately measured or improperly defined confounders may distort the obtained finding. Adjustment is also sensitive to model selection, as is outlined below. Moreover, incorrect modelling of a continuous confounder, such as the assumption of a linear relation between the confounder and the outcome, may give rise to crucial residual confounding [338]. Careful scrutiny of variable distribution and the use of restricted cubic splines and log-transformation when appropriate was undertaken to minimize this particular risk in the studies of this thesis.

What happens if an assumed confounder is, in fact, intermediate to a causal pathway between the exposure and the outcome of interest (the third criterion fails)? Adjusting for an intermediate variable removes the part of the effect of the exposure variable that is mediated by the intermediate variable. This, consequently, leads to overadjustment and a falsely reduced risk estimate for the exposure. However, this can also be used to evaluate how large a part of an exposure on the outcome is mediated through the intermediate factor [339].

Nevertheless, if an assumed confounder fails the first or second criterium but is mistakenly adjusted for anyway, this will not have large effects on the

exposure-outcome relationship. It may, however, result in a loss of statistical power and give rise to spurious associations [339].

7.4.2 Is remnant cholesterol an intermediate step on a causal pathway between apolipoprotein C-III and cardiovascular events?

We found remnant cholesterol to be a powerful predictor of CVD events in the FinnDiane cohort in the research relating to this thesis (*i.e.*, the previously unpublished data). The HR for log-transformed remnant cholesterol with respect to the composite CVD outcome was 1.61 with the most stringent level of adjustment. Besides this, we found a strong relationship between remnant cholesterol concentrations and mortality (HR 1.45).

ApoC-III was also strongly associated with the CVD outcome and all-cause mortality; however, this association lost significance when remnant cholesterol was added to the adjustment model. On the contrary, both remnant cholesterol and apoC-III were associated with the outcomes independent of LDL-cholesterol. This raises the question of whether remnant cholesterol is an intermediate step on the pathway between apoC-III and atherosclerosis; in other words, was the reason for the apoC-III risk estimate reduction after adjustment for remnant cholesterol that the effects of apoC-III are, in fact, primarily mediated through the remnants? Considering the TRL-metabolism-modulating effects of apoC-III on the role of TRL remnant cholesterol in the development of atherosclerosis, this theory is, in fact, very plausible. The CVD-risk estimate for apoC-III was, intriguingly, reduced to a non-significant level when triglycerides were accounted for in the adjustment model in the recent study by Kanter *et al.* [23]. Triglycerides *per se* are unlikely to be causal of atherosclerosis due to the reasons listed earlier. Kanter and colleagues did not assess remnant cholesterol concentrations separately; however, considering the strong correlation between remnant cholesterol and triglycerides, their finding convincingly parallels that of ours.

7.4.3 The presence and degree of diabetic kidney disease modifies the relationship between apoC-III and cardiovascular events

Remnant cholesterol might be the connecting factor between apoC-III and atherosclerosis but does not explain another angle of the results; namely, the novel discovery that the association between apoC-III and CVD is driven by individuals with albuminuria. No association was noted between apoC-III and the CVD endpoint among those who had normal AER or kidney failure in contrast to our hypothesis. *What could explain this finding*? First, it is noteworthy that dysregulated apoC-III production is a presumed link between immunosuppressive agents and secondary dyslipidemia, which is frequently encountered in individuals with immunosuppressive therapy [340,341]. It is

possible that the effects of immunosuppression on apoC-III *per se* could account for the observed disparity between the albuminuria and the kidney failure groups because a clear majority of the study participants with kidney failure had received a kidney transplant. It is also possible that the association between apoC-III and CVD in the kidney failure group was masked by the overwhelming cardiovascular risk cargo of these individuals. However, these factors do not explain the whole picture, because this disparity also extended to the normal AER group. Another possible separating factor that could explain the difference between the albuminuria and the non-albuminuria groups is endothelial dysfunction – evidently present in diabetes [342] but especially in the company of albuminuria [343]. ApoC-III is known to boost the endothelial cell expression of vascular cell adhesion molecule-1 and intercellular cell adhesion molecule-1 [287] resulting in augmented adhesion of monocytes to endothelial cells. ApoC-III has also been connected to inflammasome activation and impairment of endothelial regeneration after injury [344]. Of note, both endothelial dysfunction and inflammation are considered critical for the atherosclerosis sequence to be initiated. Thus, although remnant cholesterol seems to mediate large parts of the effects of apoC-III, the other properties of apoC-III that go beyond lipids also seem crucial.

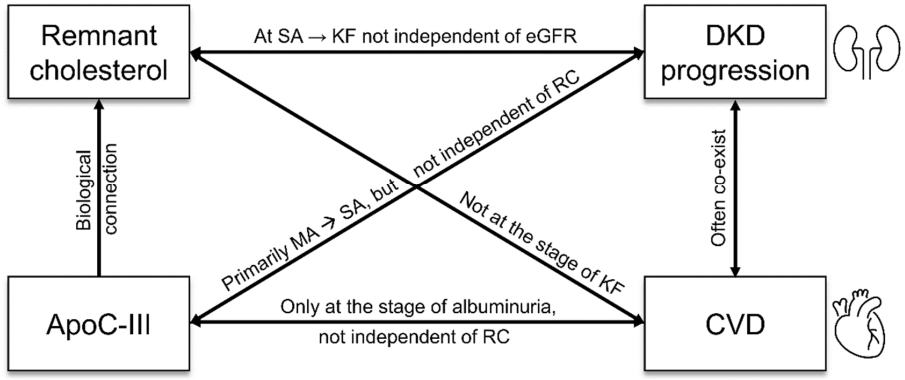


Figure 29 **A schematic picture summarizing the link between remnant cholesterol, apolipoprotein C-III, the progression of diabetic kidney disease, and cardiovascular events**. MA, moderate albuminuria; SA, severe albuminuria; RC, remnant cholesterol; KF, kidney failure.

7.5 Regression of diabetic kidney disease

We assessed the proportion of albuminuria regression among 913 FinnDiane Study participants with a history of moderate or severe albuminuria in the fourth study of this thesis. An updated classification of albuminuria was set for each participant at the first FinnDiane visit based on the three most recent albuminuria determinations (including that of the first study visit), and regression of albuminuria was defined as a reversion from a prior higher to a lower category of albuminuria. The incidence of CVD events and mortality was subsequently evaluated in relation to the regression of albuminuria.

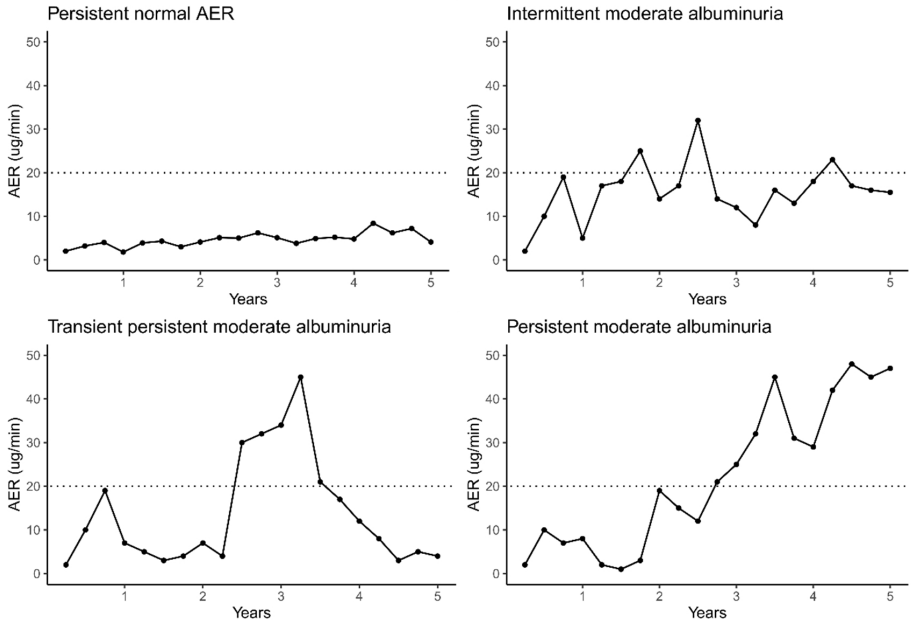


Figure 30 **Examples of the four patterns of albumin excretion rate (AER) as suggested by Steinke *et al*.** [81]**.** The black dots represent AER determinations. The dashed lines at 20 μg/min of AER mark the diagnostic threshold for moderate albuminuria. Copyright © 2005 American Diabetes Association, from Diabetes 2005;54:2164-71. Adapted with permission from the copyright holder.

7.5.1 Regression of albuminuria is a recurring phenomenon

We discovered that 23.3% of the FinnDiane Study participants with former moderate albuminuria had returned to the range of normal AER when entering the study. The regression rate from severe albuminuria to a less advanced albuminuria stage was, intriguingly, strikingly similar, namely 23.4%. The severe albuminuria regression rate was a more unpredicted

finding, because severe albuminuria has conventionally been regarded as a *point of no return* – or at least as a steadier state than moderate albuminuria. Conversely, given that the classification of the different stages of albuminuria builds on arbitrary cut-off values, the finding might not be that surprising after all.

Where does the 23% regression rate stand in relation to previous studies? Whereas some studies have found higher regression rates than this (around 50 to 60% [72,81,90,141]), regression rates in the same scope as ours, ranging between 15 and 35%, have also been published [73,74,85,345]. The proportion naturally appears to be dependent on the definition of regression as well as on the studied population. The duration of albuminuria is also crucial, because albuminuria levels tend to fluctuate especially early in the course of DKD. Four AER patterns were suggested by Steinke *et al.*, who studied the early natural history (mean initial diabetes duration 8 years) of DKD in type 1 diabetes: the persistent normal AER pattern, the intermittent moderate albuminuria pattern (single AER determinations in the moderate albuminuria area, but not meeting the two-out-of-three diagnostic criteria), the transient persistent moderate albuminuria pattern (*i.e.*, albuminuria regression), and the persistent moderate albuminuria pattern (illustration in Figure 30) [81]. It is known that, in addition to these possible patterns, some albuminuria regressors redevelop albuminuria once regression has occurred (exemplified in Figure 31) [72,74,85,91,142]. Taken together, albuminuria is a dynamic state, and we may have missed some AER fluctuations by investigating the regression status at a single time-point. However, our study population had a relatively long duration of diabetes at the baseline of the study (27.5 years; mean age 40.2 years), so our regression rate of 23% may be a better representative of the true long-term natural history than are the reports from early diabetes and early DKD.

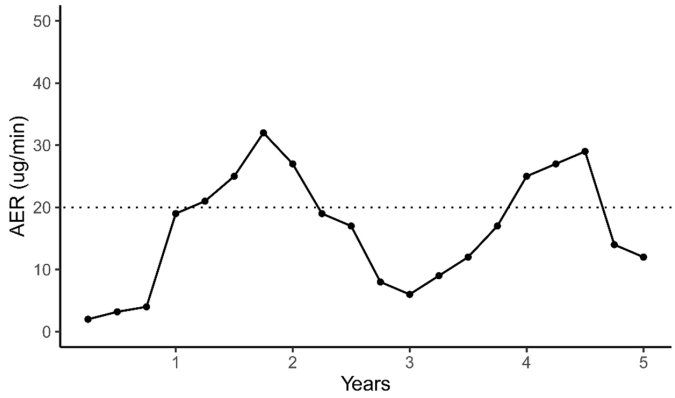


Figure 31 **A hypothetical course of AER:** the incident moderate albuminuria is followed by regression to normal AER as well as redevelopment of moderate albuminuria and its subsequent regression. These types of AER fluctuations occur especially early in the course of DKD. The black dots represent AER determinations. The dashed lines at 20 μg/min of AER mark the diagnostic threshold for moderate albuminuria.

7.5.2 Can regression of albuminuria be induced?

We further compared baseline clinical characteristics between regressors and non-regressors in Study II. The factors that differed were predominantly modifiable with lifestyle habits and/or pharmacotherapy, such as blood pressure, glycemic control, and lipids. However, these analyses need to be interpreted with caution: Despite the fact that the characteristics were measured at the first FinnDiane visit – which was also the time point of regression status determination – regression had most likely occurred before baseline in most cases.

That being said, the clinical characteristics that were significantly different between regressors and nonregressors in our cross-sectional analyses are factors that have been implicated in the progression of albuminuria (section 2.2.4). Having received intensive glycemic treatment during the DCCT conferred nearly two-fold increased chances of regression if moderate albuminuria appeared, which goes together with our independent association between regression and HbA_{1c} [142]. Therefore, although the time point of clinical characteristics determinations in our study was biased, the highlighted features could be related to regression of albuminuria after all. The first-time observed independent association between apoC-III and regression of albuminuria is intriguing, particularly considering the results that we present from Study III.

Some studies have also noted increased regression after the initiation of aggressive blood pressure-lowering treatment, especially from the stage of severe albuminuria, in type 1 diabetes [92–95]. Our cross-sectional results showed no association between the use of RAAS inhibitors and albuminuria regression; however, as the treatment guidelines encourage RAAS inhibitor initiation when moderate albuminuria appears, the finding is distorted by a high prevalence of treatment in both the regression and the nonregression groups.

7.5.3 The translation of albuminuria regression into improved prognosis

In 2022, the ADA care guidelines recommended for the first time a reduction of 30% or greater in urinary ACR in patients with diabetes and severe albuminuria to slow the progression of CKD [59]. The underlying evidence primarily stem from RAAS inhibitor studies in type 2 diabetes cohorts. Beyond the CKD outcomes, many studies have demonstrated that the presence and severity of DKD drive the cardiovascular and premature mortality risk in both type 2 [346] and type 1 diabetes [12,13]. We validated these findings in Study IV and extended them to show that the regression of albuminuria is associated with risk reduction to the level of those who did not progress in the first place. Our data are in line with findings from type 2 diabetes [96]. Reduction in

albuminuria was also observed to translate into reduced cardiovascular risk in a population with essential hypertension and left ventricular hypertrophy (88% of participants without diabetes) [347].

However, regression of albuminuria showed no significant cardiovascular benefit in the DCCT/EDIC cohort of individuals with type 1 diabetes. The discrepancy between the DCCT/EDIC finding and ours is confusing, because many factors in the study designs are alike, for example, the comparable cohort sizes and the analogous endpoint definitions. The follow-up was some years longer in the DCCT/EDIC study; hence, larger differences between the regressors and nonregressors in the FinnDiane cohort may occur over time. However, our Kaplan-Meier analyses indicate that the differences in survival appeared very quickly and remained as such for the 15 years of follow-up. The DCCT/EDIC did not present survival/cumulative incidence over time, so we were unable to compare this feature between the studies.

Factors that, conversely, could have contributed to the observed disparities are the definition of regression (our single time-point definition vs. the time-updated in the other cohort) and some dissimilarities in study populations, for instance, that the FinnDiane participants were older, had a longer diabetes duration, and had higher blood pressure at baseline. Furthermore, the results originate from very differently designed studies, namely from a multicenter observational study (the FinnDiane) *vs.* an intervention study (the DCCT). Therefore, our novel observations may be considered to apply to individuals with type 1 diabetes in general.

We noted higher eGFR concentrations among the regressors when separately analyzing the FinnDiane Study participants with a history of severe albuminuria. eGFR is a very potent predictor of mortality risk; thus, we hypothesized that the difference in survival is mainly attributable to kidney function. However, to our surprise, the multivariable regression analyses revealed that the association between albuminuria regression and mortality is independent of eGFR and of other well-established risk factors, such as HbA_{1c} and systolic blood pressure. In other words, the improvements in kidney function, glycemic control, and blood pressure, which seem to co-occur with AER improvement, do not fully explain the translation of regression into improved prognosis. What the missing mediator might be warrants further investigation.

7.6 Future scopes of research

This thesis answered many questions related to the incidence, progression, and regression of DKD in type 1 diabetes; however, it has also engendered some new ones, and some of its covered subjects will need further elucidation.

First, the proportion and rate of albuminuria regression should be assessed in a larger, preferably population-based cohort (*e.g.,* the cohort of Study I) in the future. It would be particularly interesting, in view of the advances in DKD

screening and treatment, to investigate whether this rate has changed over time. Our results on a connection between regression of albuminuria and diminished risk of CVD events and mortality contrasted with some other previous research, so this would also be important to restudy in another population.

Furthermore, the cumulative incidences of both moderate and severe albuminuria should be reassessed in the 1980-99 cohort – or some other corresponding study population – when a longer follow-up has been attained. It would be interesting to see whether the changes in the incidence patterns we report are specific to the Finnish population or whether this finding can also be extended to other populations.

This thesis introduced the TRL metabolism to the context of DKD in type 1 diabetes, but more research is undoubtedly needed to elucidate this relationship further. Regarding the TRL metabolism, a potential future wider scope of treatment indications for *Volanesorsen*, a specific apoC-III inhibitor, is awaited. If individuals with type 1 diabetes are included in the drug studies with *Volanesorsen* at some point, the effects in those with concomitant albuminuria would be particularly interesting, considering that the association between apoC-III and macrovascular disease was limited to this group of individuals in our Study III.

8 SUMMARY AND CONCLUSIONS

I The incidence rate pattern of severe albuminuria had changed over time, as the observed incidence peak at 15-19 years since diabetes onset in the 1970-79 cohort was not replicated in those diagnosed later. The cumulative incidence of severe albuminuria had approximately halved between the 1970s and 1980s, whereas no further improvement was noted after this. The cumulative incidence of moderate albuminuria showed no signs of decline between the 1980s and the 1990s. Furthermore, the results revealed that the progression rate from severe albuminuria to kidney failure had remained unaffected. The lowest cumulative risk of DKD was seen in those with early-onset type 1 diabetes, whereas a sex difference in the risk was only apparent if diabetes had been diagnosed at the age of 10 or later.

II Remnant cholesterol concentration was robustly associated with every step of DKD progression except for that between severe albuminuria and kidney failure, when the relationship was not independent of eGFR. The variability of remnant cholesterol was not associated with the progression of DKD in adjusted analyses. ApoC-III was associated with the progression of moderate to severe albuminuria yet not independent of remnant cholesterol. The association between apoC-III and progression to moderate albuminuria was most weakened by HbA_{1c} at the stage of normal AER. The association between apoC-III and progression to kidney failure was most weakened by eGFR at the stage of severe albuminuria.

III Remnant cholesterol concentration was a powerful predictor of future CVD events and all-cause mortality except in the study participants with kidney failure. The association between apoC-III and the outcomes was limited to those with albuminuria. The point estimate for apoC-III was also reduced to a nonsignificant level in the albuminuria group when remnant cholesterol was added to the adjustment models, suggesting that remnant cholesterol is the mediator of the effects of apoC-III.

IV The albuminuria regression rate was 23% in the FinnDiane cohort independent of the initial stage of DKD (moderate or severe albuminuria). Regression of albuminuria was associated with a risk reduction of CVD events to the level of those who did not progress in the first place. The same phenomenon was observed for all-cause mortality. Regression of albuminuria was associated with female sex, lower HbA_{1c}, and lower concentrations of apoC-III in a cross-sectional analysis.

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Helsinki, May 2022
Fanny Jansson Sigfrids

APPENDIX

FinnDiane Study Centers	Physicians and nurses
Anjalankoski Health Centre	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 Länsi-Pohja, Kemi	H. Laukkanen, P. Nyländen, A. Sademies
Central Ostrabothnian Hospital District, Kokkola	S. Anderson, B. Asplund, U. Byskata, P. Liedes, M. Kuusela, T. Virkkala
City of Espoo Health Centre	
Espoonlahti	A. Nikkola, E. Ritola
Tapiola	M. Niska, H. Saarinen
Samaria	E. Oukko-Ruponen, T. Virtanen
Viherlaakso	A. Lyytinen
City of Helsinki Health Centre	
Puistola	H. Kari, T. Simonen
Suutarila	A. Kaprio, J. Kärkkäinen, B. Rantaeskola
Töölö	P. Kääriäinen, J. Haaga, A-L. Pietiläinen
City of Hyvinkää Health Centre	S. Klemetti, T. Nyandoto, E. Rontu, S. Satuli-Autere
City of Vantaa Health Centre	
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 Centre	P. Hentunen, J. Lagerstam
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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 Centre, Kyllö	K. Nuorva, M. Tiihonen
Kainuu Central Hospital, Kajaani	S. Jokelainen, P. Kemppainen, A-M. Mankinen, M. Sankari
Kerava Health Centre	H. Stuckey, P. Suominen

Kirkkonummi Health Centre	A. Lappalainen, M. Liimatainen, J. Santaholma
Kivelä Hospital, Helsinki	A. Aimolahti, E. Huovinen
Koskela Hospital, Helsinki	V. Ilkka, M. Lehtimäki
Kotka Heath Centre	E. Pälikkö-Kontinen, A. Vanhanen
Kouvola Health Centre	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 Centre	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 Centre	P. Linkola, I. Pulli
Lohja Hospital	T. Granlund, M. Saari, T. Salonen
Loimaa Health Centre	A. Mäkelä, P. Eloranta
Länsi-Uusimaa Hospital, Tammisaari	I-M. Jousmaa, J. Rinne
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
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Oulu Health Centre	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 Centre	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	E. Korpi-Hyövälti, T. Latvala, E. Leijala
South Karelia Central Hospital, Lappeenranta	T. Ensala, E. Hussi, R. Härkönen, U. Nyholm, J. Toivanen
Tampere Health Centre	A. Vaden, P. Alarotu, E. Kujansuu, H. Kirkkopelto-Jokinen, M. Helin, S. Gummerus, L. Calonius, T. Niskanen, T. Kaitala, T. Vatanen
Tampere University Hospital	I. Ala-Houhala, T. Kuningas, P. Lampinen, M. Määttä, H. Oksala, T. Oksanen, K. Salonen, H. Tauriainen, S. Tulokas

Tiirismaa Health Centre, Hollola	T. Kivelä, L, Petlin, L. Savolainen
Turku Health Centre	I. Hämäläinen, H. Virtamo, M. Vähätalo
Turku University Central Hospital	K. Breitholz, R. Eskola, K. Metsärinne, U. Pietilä, P. Saarinen, R. Tuominen, S. Äyräpää
Vaajakoski Health Centre	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
Vaasa Central Hospital	S. Bergkulla, U. Hautamäki, V-A. Myllyniemi, I. Rusk

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