Lipid profile and micro- and macrovascular complications in type 1 diabetes

Nina Tolonen

ACADEMIC DISSERTATION

To be presented,
with the permission of the Medical Faculty of the University of Helsinki,
for public examination in Auditorium 2, Biomedicum Helsinki,
on January 23rd 2015, at 12 noon.

Helsinki 2015
To my family, friends, colleagues, and all FinnDiane patients

He who studies medicine without books
sails an uncharted sea,
but he who studies medicine without patients
does not go to sea at all.

Sir William Osler
CONTENTS

LIST OF ORIGINAL PUBLICATIONS ................................................................. 7
ABBREVIATIONS .......................................................................................... 8
ABSTRACT ...................................................................................................... 10
TIIVISTELMÄ (ABSTRACT IN FINNISH) ...................................................... 12
ABSTRAKT (ABSTRACT IN SWEDISH) ........................................................ 14
1 INTRODUCTION ......................................................................................... 16
2 REVIEW OF THE LITERATURE ................................................................. 18
   2.1 Types of diabetes mellitus ................................................................. 18
      2.1.1 Definition of diabetes ................................................................. 18
      2.1.2 Classification of diabetes ............................................................. 18
      2.1.3 Epidemiology of type 1 diabetes ............................................... 20
      2.1.4 Pathogenesis of type 1 diabetes ............................................... 20
   2.2 Diabetic complications ................................................................. 22
      2.2.1 Diabetic nephropathy ................................................................. 22
         2.2.1.1 Definition ................................................................. 22
         2.2.1.2 Renal function ............................................................. 22
         2.2.1.3 Epidemiology ............................................................. 23
         2.2.1.4 Pathogenesis ............................................................... 24
         2.2.1.5 Risk factors ................................................................. 25
      2.2.2 Diabetic retinopathy ................................................................. 28
         2.2.2.1 Epidemiology ............................................................... 29
         2.2.2.2 Risk factors ................................................................. 30
      2.2.3 Diabetic neuropathy ................................................................. 31
      2.2.4 Macrovascular complications ............................................... 32
         2.2.4.1 Coronary artery disease ............................................... 33
   2.3 Lipoprotein metabolism ................................................................. 34
      2.3.1 Actions of insulin on lipoprotein metabolism ......................... 36
      2.3.2 Insulin resistance and lipoprotein metabolism ....................... 37
      2.3.3 Apolipoproteins in diabetes ................................................... 39
      2.3.4 Secondary changes in lipid profile in nephrotic syndrome ....... 40
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:


*equal contribution

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACCORD</td>
<td>Action to Control Cardiovascular Risk in Diabetes</td>
</tr>
<tr>
<td>ACE</td>
<td>Angiotensin-converting enzyme</td>
</tr>
<tr>
<td>ACR</td>
<td>Albumin-to-creatinine ratio</td>
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<tr>
<td>AdDIT</td>
<td>Adolescent type 1 Diabetes, cardio-renal Intervention Trial</td>
</tr>
<tr>
<td>ADVANCE</td>
<td>Action in Diabetes and Vascular Disease</td>
</tr>
<tr>
<td>AER</td>
<td>Albumin excretion rate in urine</td>
</tr>
<tr>
<td>AGE</td>
<td>Advanced glycation end-product</td>
</tr>
<tr>
<td>ALERT</td>
<td>Assessment of Lescol in Renal Transplantation</td>
</tr>
<tr>
<td>ALLHAT-LLT</td>
<td>Lipid-Lowering Treatment to Prevent Heart Attack Trial - Lipid-Lowering Trial</td>
</tr>
<tr>
<td>Apo</td>
<td>Apolipoprotein</td>
</tr>
<tr>
<td>ARB</td>
<td>Angiotensin II receptor blocker</td>
</tr>
<tr>
<td>ARIC</td>
<td>Atherosclerosis Risk in Communities</td>
</tr>
<tr>
<td>ASCOT-LLA</td>
<td>Anglo-Scandinavian Cardiac Outcomes Trial - Lipid-Lowering Arm</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CAN</td>
<td>Cardiovascular autonomic neuropathy</td>
</tr>
<tr>
<td>CARDS</td>
<td>Collaborative Atorvastatin Diabetes Study</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CKD</td>
<td>Chronic kidney disease</td>
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<tr>
<td>CKD-EPI</td>
<td>Chronic Kidney Disease Epidemiology Collaboration</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DAIS</td>
<td>Diabetes Atherosclerosis Intervention Study</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>DCCT</td>
<td>Diabetes Control and Complications Trial</td>
</tr>
<tr>
<td>DCCT/EDIC</td>
<td>Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study</td>
</tr>
<tr>
<td>DPN</td>
<td>Distal symmetric polyneuropathy</td>
</tr>
<tr>
<td>FIELD</td>
<td>Fenofibrate Intervention and Event Lowering in Diabetes</td>
</tr>
<tr>
<td>FinnDiane</td>
<td>Finnish Diabetic Nephropathy Study</td>
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<tr>
<td>eGDR</td>
<td>Estimated glucose disposal rate</td>
</tr>
<tr>
<td>eGFR</td>
<td>Estimated glomerular filtration rate</td>
</tr>
<tr>
<td>ERFC</td>
<td>Emerging Risk Factor Collaboration</td>
</tr>
<tr>
<td>ESRD</td>
<td>End-stage renal disease</td>
</tr>
<tr>
<td>ETDRS</td>
<td>Early Treatment of Diabetic Retinopathy Study</td>
</tr>
<tr>
<td>GADA</td>
<td>Glutamic acid decarboxylase antibodies</td>
</tr>
<tr>
<td>GENIE</td>
<td>Genetics of Nephropathy - an International Effort</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycosylated hemoglobin A1c</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>HL</td>
<td>Hepatic lipase</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
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<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
</tr>
<tr>
<td>HMG-CoA</td>
<td>3-Hydroxy-3-methylglutaryl coenzyme A</td>
</tr>
<tr>
<td>HPS</td>
<td>Heart Protection Study</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard ratio</td>
</tr>
<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
</tr>
<tr>
<td>IDL</td>
<td>Intermediate-density lipoprotein</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>JUPITER</td>
<td>Justification for the Use of Statins in Prevention - an Intervention Trial Evaluating Rosuvastatin</td>
</tr>
<tr>
<td>LADA</td>
<td>Latent Autoimmune Diabetes of the Adult</td>
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<tr>
<td>LCAT</td>
<td>Lecithin–cholesterol acyltransferase</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>Ln</td>
<td>Natural logarithm</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>Lipoprotein(a)</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
</tr>
<tr>
<td>MDRD</td>
<td>Modification of Diet in Renal Disease</td>
</tr>
<tr>
<td>MODY</td>
<td>Maturity onset diabetes of the young</td>
</tr>
<tr>
<td>NNT</td>
<td>Number needed to treat</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NPDR</td>
<td>Non-proliferative diabetic retinopathy</td>
</tr>
<tr>
<td>NRI</td>
<td>Net reclassification improvement</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>oxLDL</td>
<td>Oxidized LDL</td>
</tr>
<tr>
<td>PDR</td>
<td>Proliferative diabetic retinopathy</td>
</tr>
<tr>
<td>Pittsburgh EDC</td>
<td>Pittsburgh Epidemiology of Diabetes Complications</td>
</tr>
<tr>
<td>PLTP</td>
<td>Phospholipid transfer protein</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver-operating characteristic</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>sdLDL</td>
<td>Small, dense LDL</td>
</tr>
<tr>
<td>Swedish NDR</td>
<td>Swedish National Diabetes Register</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Transforming growth factor beta</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
</tr>
<tr>
<td>TNT</td>
<td>Treating to New Targets</td>
</tr>
<tr>
<td>TRL</td>
<td>Triglyceride-rich lipoprotein</td>
</tr>
<tr>
<td>UKPDS</td>
<td>United Kingdom Prospective Diabetes Study</td>
</tr>
<tr>
<td>VADT</td>
<td>Veterans Affairs Diabetes Trial</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very-low-density lipoprotein</td>
</tr>
<tr>
<td>WESDR</td>
<td>Wisconsin Epidemiologic Study of Diabetic Retinopathy</td>
</tr>
<tr>
<td>WHO-MSVDD</td>
<td>World Health Organization Multinational Study of Vascular Disease in Diabetes</td>
</tr>
<tr>
<td>WHR</td>
<td>Waist-to-hip ratio</td>
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</tbody>
</table>
ABSTRACT

Background
Cardiovascular disease is the most common cause of death in patients with type 1 diabetes (1), and the premature mortality rates are especially high in patients with diabetic nephropathy (2, 3). Diabetic retinopathy is the leading cause of vision loss among the working-age population in industrialized countries (4). Early identification and aggressive treatment of risk factors are crucial to reduce the incidence of diabetic complications.

Aims
To examine the relationships between lipid profiles and diabetic nephropathy, diabetic retinopathy, and incident coronary artery disease (CAD) events in a large nationwide cohort of patients with type 1 diabetes.

Subjects and methods
These studies are part of the ongoing Finnish Diabetic Nephropathy Study (FinnDiane), a nationwide, multicenter study aimed at identifying both genetic and clinical risk factors for the development of diabetic complications in patients with type 1 diabetes. Studies I (N=2927) and III (N=1465) have a cross-sectional design. At follow-up, renal status was verified by a review of all available medical files, including laboratory data (Study II, N=2304), and data on CAD events were retrieved from the Finnish Hospital Discharge Register and the Causes of Death Register (Study IV, N=3520). Ophthalmologic data from fundus photographs and ophthalmic records were graded with the Early Treatment of Diabetic Retinopathy Study (ETDRS) scale (Study III).

Results
Triglycerides and apolipoprotein (Apo) B were independently associated with estimated glomerular filtration rate (eGFR) in the multivariate models. The recommended lipid concentrations of current treatment guidelines were poorly met, especially regarding the target for LDL cholesterol. Triglycerides and ApoB were independent predictors of progression to micro- and macroalbuminuria, and total cholesterol was an independent predictor of progression to end-stage renal disease. HDL and HDL2 cholesterol were independently associated with proliferative diabetic retinopathy (PDR), and triglycerides and triglyceride/HDL cholesterol ratio with mild non-proliferative diabetic retinopathy (NPDR). In patients with moderate to severe NPDR or PDR, the correlations between albumin excretion rate (AER) and lipid variables were strong. However, in patients without retinopathy no significant correlations were observed. In multivariate models, ApoB, triglycerides, non-HDL cholesterol, ApoB/ApoA-I ratio, and triglyceride/HDL cholesterol ratio were the strongest lipid predictors of an incident CAD event.
Conclusions
Lipid abnormalities were associated with an increased risk of all three diabetic complications studied, i.e. diabetic nephropathy, retinopathy, and incident CAD events. Triglycerides and ApoB were independently associated with AER and eGFR and predicted the progression to micro- and macroalbuminuria as well as incident CAD events. Far lower concentrations of triglycerides than the currently recommended cut-off level (<1.7 mmol/l) increased the risk of progression of renal disease and predicted incident CAD events. Total and LDL cholesterol were poor predictors of an incident CAD event in patients with normal AER, in patients with HbA1c below the median of the cohort, and in women, in whom the ratios of atherogenic and anti-atherogenic lipoproteins and lipids performed better. Current treatment recommendations may need to be revised to reflect residual CAD risk in patients with type 1 diabetes.
TIIVISTELMÄ (ABSTRACT IN FINNISH)

Tausta
Sydän- ja verisuonitaudit ovat yleisin kuolinsyy tyypin 1 diabeetikoilla (1), ja ennenaikainen kuolleisuus on erityisen suuri potilailla, joilla on diabeettinen munuaistautu eli nefropatia (2, 3). Diabetekseen liittyvä silmäsairaus, retinopatia, on sokeutumisen yleisin syy länsimaiden työväenluostareiden väestössä (4). Riskitekijöiden varhainen tunnistaminen ja tehokas hoito ovat ratkaisevassa asemassa jotta voisimme vähentää diabeettisten liitännäissairauksien syntyä.

Tavoitteet
Tavoitteena oli tutkia veren rasva-arvojen ja diabeettisen nefropatian, retinopatian, ja sepelvaltimotaipapan tapahtumien (ensimmäinen sydäninfarkti, sepelvaltimoiden pallolaajennus tai ohitusleikkaus) yhteyttä suurra valtakunnallisessa tyypin 1 diabetes-populaationissa.

Aineisto ja menetelmät
Tutkimukset ovat osa FinnDiane-tutkimusta (Finnish Diabetic Nephropathy Study), jonka tavoitteena on selvittää tyypin 1 diabeteksen liitännäissairauksien geneettisiä ja kliinisiä riskitekijöitä. Osatyö I (N = 2927) ja III (N = 1465) ovat poikkileikkaustutkimuksia. Seurantatutkimuksissa tieto munuaistaudin vaikeusasteesta varmennettiin kaikista käytettävissä olevista sairaskeromuksista (osatyö II, N = 2304), ja tiedot sepelvaltimotaipapapeita saatiin hoitoilmoitusjärjestelmästä (HILMO) sekä kuolinsyyrekisteristä (osatyö IV, N = 3520).

Tulokset
Päätelmät

ABSTRAKT (ABSTRACT IN SWEDISH)

Bakgrund
Hjärt-och kärlsjukdomar är den vanligaste dödsorsaken bland typ 1 diabetiker (1), och den förtidiga dödligheten är särskilt hög hos patienter med diabetisk njursjukdom, nefropati (2, 3). Diabetisk ögonsjukdom, retinopati, är den vanligaste orsaken till blindhet bland den arbetsföra befolkningen i västvärlden (4). En tidig identifiering och effektiv behandling av riskfaktorer har en avgörande betydelse för att minska förekomsten av följdsjukdomar vid typ 1 diabetes.

Studiens målsättningar
Att undersöka sambandet mellan lipidprofiler och diabetisk nefropati, retinopati och kranskärlssjukdom (första hjärtinfarkt, ballongutvidgning eller bypassoperation av hjärtats kransväv) i en stor landsomfattande cohort av patienter med typ 1 diabetes.

Patienter och metoder
Dessa studier är en del av den pågående FinnDiane-studien (Finnish Diabetic Nephropathy Study), en landsomfattande, multicenterstudie vars målsättning är att identifiera både genetiska och kliniska riskfaktorer för utvecklingen av följdsjukdomar vid typ 1 diabetes. Studie I (N = 2927) och III (N = 1465) var tvärsnittsstudier. Vid uppföljningen verifierades progression av njursjukdom genom en granskning av alla tillgängliga sjukjournaler (studie II, N = 2304) och uppgifter om kranskärlssjukdom söktes ur Finlands patient- och dödsorsaksregister (studie IV, N = 3520).

Resultat
**Slutsatser**

En ofördelaktig lipidprofil var associerad med samtliga av de tre undersökta diabeteskomplikationerna, dvs. diabetisk nefropati, retinopati, och kranskärlssjukdom. Förhöjda triglycerid- och ApoB-nivåer förutspådde både progression av njursjukdom samt insjuknande i kranskärlssjukdom. Betydligt lägre koncentrationer av triglycerider än den för tillfället rekommenderade nivån (<1.7 mmol/l) ökade risken för progression av njursjukdom och insjuknande i kranskärlssjukdom bland typ 1 diabetiker. När patienterna delades in i grupper på basen av kön, graden av njursjukdom eller sockerbalans, förutspådde inte total- och LDL-kolesterolnivåerna kranskärlssjukdom hos kvinnor, patienter med normalt AER, eller patienter med HbA1c under 8.3 %. Förhållandet mellan de aterogena och anti-aterogena lipiderna var betydligt bättre prediktorer för kranskärlssjukdom hos dessa patienter. Gängse behandlingsrekommendationer för typ 1 diabetiker bör eventuellt revideras för att bättre upptäcka den potentiellt ökade kranskärlssjukdomsrisken.
1 INTRODUCTION

Diabetes is one of the most common chronic diseases worldwide, and in 2013 altogether 382 million people were estimated to have diabetes (5). The global prevalence of type 2 diabetes is increasing in epidemic proportions due to an increase in obesity, a low level of physical activity, and aging of the population. In 2035 a predicted 592 million people will have diabetes (5). In Finland, ~250 000 individuals have diagnosed type 2 diabetes and ~200 000 are estimated to have undiagnosed type 2 diabetes (6). It is noteworthy that Finland has the highest incidence of type 1 diabetes in the world (7), with the current number of patients with type 1 diabetes being ~50 000 (8). The incidence of type 1 diabetes is also increasing worldwide, but the reasons for this remain unclear (9).

Type 1 diabetes was a fatal disease until the discovery of insulin in 1921. Despite modern insulin treatment, glycemic control is poor in many patients with type 1 diabetes, and in the Finnish Diabetic Nephropathy Study (FinnDiane) cohort only ~15% of the patients had reached the recommended glycosylated hemoglobin A$_{1c}$ (HbA$_{1c}$) level of <7.0% (10). With increasing HbA$_{1c}$, the frequency of diabetic complications also increases substantially. Diabetic complications lead to reduced quality of life and premature death. The management and treatment of these complications also cause an immense economic burden (11).

Diabetic kidney disease (nephropathy) is the leading cause of dialysis or kidney transplantation (12). Nephropathy develops in about one-third of patients with type 1 diabetes, and the highest incidence peak is seen after 15-20 years of diabetes (13, 14). Diabetic eye disease (retinopathy) is the most common cause of blindness among the working-aged population in the Western world (4). Proliferative diabetic retinopathy (PDR), the advanced form of diabetic retinopathy, occurs in around 40% of patients with type 1 diabetes after 25 years of diabetes duration (15). Diabetic nephropathy and retinopathy share several risk factors and are strongly associated with each other (16). The studies regarding the association between lipid variables and retinopathy have yielded conflicting results (17-20), and the effect of renal disease on this relationship is unclear. Also unknown is how retinopathy status affects the association between albumin excretion rate (AER) and lipid variables.

The most common cause of death in patients with type 1 diabetes is cardiovascular disease (CVD) (21). The increased incidence of CVD in patients with type 1 diabetes is mostly related to renal disease, and the mortality rates of patients with type 1 diabetes without any signs of renal disease are comparable with those of the general population, whereas in patients with end-stage renal disease (ESRD) an 18- to 30-fold increase in mortality has been observed (2, 3). The role of the lipid variables in the development of coronary artery disease (CAD) has been studied thoroughly in the general population and in patients with type 2 diabetes, but studies in patients with type 1 diabetes are scarce.
The main aim of these series of studies was to evaluate the relationship between lipid profiles and different diabetic complications, i.e. diabetic nephropathy, retinopathy, and incident CAD events, in a large nationwide cohort of patients with type 1 diabetes.
2 REVIEW OF THE LITERATURE

2.1 Types of diabetes mellitus

2.1.1 Definition of diabetes

Diabetes mellitus is a chronic systemic disease characterized by an increased blood glucose concentration. The word diabetes is derived from the Greek word “diabainein” and means “to pass through”, referring to the large volume of urine, while mellitus comes from the Latin term “mel”, which means honey and refers to the sweetness of the urine from patients with untreated diabetes. Diabetes is caused by either decreased production of insulin from the pancreatic β-cells or decreased effect of insulin on target tissues or by a combination of these two. Diabetes not only causes disturbances in carbohydrate metabolism, but also affects lipid and protein metabolisms. Diabetes is defined as an increased fasting plasma glucose $\geq 7.0$ mmol/l or a 2-h plasma glucose $\geq 11.1$ mmol/l during an oral glucose tolerance test or HbA$_1c$ $\geq 6.5\%$ or a random plasma glucose of $\geq 11.1$ mmol/l in a patient with classic symptoms of hyperglycemia (thirst, weight loss, and polyuria) (22-24).

2.1.2 Classification of diabetes

The two major categories of diabetes are type 1 and type 2 diabetes, previously also called “insulin-dependent” (IDDM) and “non-insulin-dependent” (NIDDM), or “juvenile” and “adult-onset” diabetes, respectively. Type 1 diabetes is characterized by an autoimmune reaction that leads to a total loss of function of the insulin-secreting β-cells of the islets of Langerhans in the pancreas, resulting in absolute insulin deficiency. Type 2 diabetes is the consequence of decreased insulin sensitivity (primarily in skeletal muscles, adipose tissue, and liver) and/or decreased insulin secretion from β-cells. It is the most common form of diabetes and is increasing in epidemic proportions worldwide. Considerable overlap exists between the two conditions, and type 1 and type 2 diabetes have been proposed to be different forms of the same disease, the main difference being the absence of an immune response in patients with type 2 diabetes, leading to a slower rate of β-cell loss (25). On the other hand, the clear lack of evidence for similar genetic factors predisposing to type 1 and type 2 diabetes supports the notion of two separate diseases.

Latent Autoimmune Diabetes of the Adult (LADA) is classified as a form of type 1 diabetes and is characterized by the presence of islet autoantibodies, most typically glutamic acid decarboxylase antibodies (GADA), leading to the destruction of pancreatic β-cells (26, 27). Patients with LADA are usually older than the patients with type 1 diabetes and do not require insulin at the time of diagnosis, but after 5 years of diabetes
duration ~80% require insulin treatment. The clinical phenotype resembles that of type 2 diabetes, and LADA patients may initially be diagnosed as having type 2 diabetes. Due to the features described above, LADA is sometimes also called “type 1.5 diabetes” or “slow-onset type 1 diabetes”.

Maturity onset diabetes of the young (MODY) is a heterogeneous group of disorders caused by mutations in different autosomal dominant genes with high penetration, affecting insulin production or insulin release from pancreatic β-cells (28). It can also be referred to as monogenic diabetes, in contrast to the more complex type 1 and type 2 diabetes, which involve multiple genes with low penetration as well as environmental factors. MODY patients do not display the β-cell autoimmunity or ketoacidosis experienced by patients with type 1 diabetes, and the age at onset is usually younger than in patients with type 2 diabetes. Typical characteristics of type 1 and type 2 diabetes, LADA, and MODY can be seen in Table 1.

Table 1. Typical characteristics of different forms of diabetes.

<table>
<thead>
<tr>
<th></th>
<th>Type 1 diabetes</th>
<th>Type 2 diabetes</th>
<th>LADA</th>
<th>MODY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at onset</strong></td>
<td>Usually age below 35 years</td>
<td>Usually in adults over 40 years</td>
<td>Usually age above 30 years</td>
<td>Usually age below 30 years</td>
</tr>
<tr>
<td><strong>Characteristics of diagnosis</strong></td>
<td>Acute, often ketosis</td>
<td>Often insidious</td>
<td>Symptoms develop more slowly than in type 1 diabetes</td>
<td>Variable, many are asymptomatic</td>
</tr>
<tr>
<td><strong>Insulin level at diagnosis</strong></td>
<td>Undetectable or very low</td>
<td>High</td>
<td>Low</td>
<td>Variable</td>
</tr>
<tr>
<td><strong>Presence of insulin resistance</strong></td>
<td>Usually no (but may be present in obese patients)</td>
<td>Yes</td>
<td>Yes (in some studies less common than in type 2 diabetes)</td>
<td>No (insulin resistance is extremely rare)</td>
</tr>
<tr>
<td><strong>Insulin therapy</strong></td>
<td>Essential and permanent</td>
<td>May occur</td>
<td>Usually not at diagnosis, most need insulin within 5 years</td>
<td>May occur (insulin doses are low)</td>
</tr>
<tr>
<td><strong>Autoantibodies</strong></td>
<td>IAA, GADA, ICA, IA2-A, ZnT8A...</td>
<td>None</td>
<td>Mostly GADA and ICA (IA-2A, IAA, ZnT8A may be detected)</td>
<td>None/Rare</td>
</tr>
<tr>
<td><strong>Genetics</strong></td>
<td>Polygenic</td>
<td>Polygenic</td>
<td>Polygenic</td>
<td>Monogenic</td>
</tr>
<tr>
<td><strong>Autoimmune etiology</strong></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

IAA=insulin autoantibodies, GADA=glutamic acid decarboxylase antibodies, ICA=islet cell antibodies, IA2-A=insulinoma-associated autoantigen 2 antibodies, ZnT8A = Zink transporter 8 antibodies.

Gestational diabetes is diagnosed when hyperglycemia is first recognized during pregnancy and is associated with insulin resistance and increased risk of type 2 diabetes (29).
Secondary forms of diabetes can be caused by, for example, pancreatitis, surgery, pancreatic trauma, and pancreatic cancer. Further, long-term use of such medications as steroids, antipsychotics, and a range of immunosuppressive agents may induce the development of diabetes (30). Medications widely used for prevention of CVD, e.g. thiazide diuretics, beta-blockers, and statins have also been found to be weakly diabetogenic (30, 31).

2.1.3 Epidemiology of type 1 diabetes

Type 1 diabetes is one of the most common chronic diseases of childhood, and in contrast to other autoimmune diseases, it has a male predominance (32). The highest incidence of type 1 diabetes is found in Finland (64 per 100 000 per year below the age of 15) (7), followed by the Italian island of Sardinia and Sweden (33). The lowest incidence of type 1 diabetes is found in Venezuela and China, with only around 0.1 per 100 000 per year (33). The incidence of type 2 diabetes is increasing rapidly, mainly because of sedentary lifestyle and an increase in obesity, but the reasons behind the worldwide increase in the incidence of type 1 diabetes remain unclear. In Finland, a non-linear increase has been observed, and the incidence rate of type 1 diabetes in Finnish children has doubled from 1980 to 2005 (7). Recent studies have reported a plateau in the incidence rates in Finland and Sweden (34, 35). Another recent finding is that the onset of diabetes has shifted towards a younger age (36, 37), and according to the “spring harvest theory” the increasing incidence in children might be compensated by a decrease in the incidence in the older age groups (38). In concordance with this theory, the overall cumulative incidence of type 1 diabetes before the age of 39 years in Sweden and Belgium has remained constant (37, 39). However, in Finland the increasing incidence of type 1 diabetes is also seen in the age group of 15-39 years (40); thus, in Finland the “spring harvest theory” does not seem to apply. Genetic variation is thought to be one explanation for the marked geographical differences in the incidence rates, but genetic changes and more children being borne to parents with type 1 diabetes are still insufficient to explain the increased incidence. Thus, environmental factors must play a role. In support of this, increased incidence of type 1 diabetes has also been reported in children of migrants who have moved from a region of low to high incidence of type 1 diabetes (41, 42).

2.1.4 Pathogenesis of type 1 diabetes

Type 1 diabetes involves selective β-cell loss and is the result of an autoimmune reaction. T-cells (CD8+ and CD4+), macrophages (CD68+), and B-lymphocytes (CB20+) are frequently found in insulitis lesions (43). Autoantibodies against β-cell autoantigens, e.g. insulin autoantibodies (IAA), GADA, islet cell antibodies (ICA), insulinoma-associated autoantigen 2 antibodies (IA-2A), and Zink transporter 8 antibodies (ZnT8A) (44), are found in more than 90% of patients with newly diagnosed type 1 diabetes and they are
already present months to years before symptomatic onset. However, only 25-50% of children with autoantibody positivity will eventually develop clinical type 1 diabetes, hence, many remain in a subclinical state or the β-cell autoimmunity is aborted (45). Type 1 diabetes is a polygenic disease, and thus far, over 40 loci are known to affect susceptibility to the disease (46, 47). Most of these loci are believed to involve immune responses. The highest risk is associated with the human leukocyte antigen (HLA) region on chromosome 6p21 (48), which accounts for ~50% of the genetic susceptibility. However, the proportion of high-risk HLA genotypes in newly diagnosed patients has decreased, and therefore, the influence of the environment is thought to have increased (49). The true triggers of the autoimmune reaction in genetically susceptible individuals remain obscure. High birth weight and weight gain in infancy have been suggested as risk factors for type 1 diabetes, and in the “accelerator hypothesis” increased body mass is thought to overload the β-cells, with the increased insulin demand accelerating the autoimmune attack (25). Different dietary factors, such as cow milk (50), potatoes infested by Streptomyces species (51), gluten (52), and short duration of breast feeding (53), have been suggested as environmental agents initiating the disease process. Other triggers might be vitamin D deficiency (54, 55), enteroviruses (56), Cesarean section (57), and gut microbiota (58). Interestingly, seasonal changes in incidence rates have also been noted. Being born in the spring is associated with a higher risk (59), and more cases are diagnosed in the fall and winter (60) at the peak occurrence of enteroviruses and vitamin D deficiency. The “hygiene hypothesis” suggests that a decrease in infections during childhood leads to inadequate functioning of the immune system (61). In support of this theory, a reciprocal trend has been seen between the incidence of infectious diseases and the incidence of autoimmune and allergic diseases. Furthermore, the incidence of type 1 diabetes is positively associated with the gross national product (62) and is lower in poor countries with a higher population density (63). Also, a protective effect of exposure to infections with early daycare attendance has been observed (64). Interestingly, metabolite and lipid profiles have also been suggested as markers for the development of type 1 diabetes. For example, reduced phosphatidylcholine at birth, and decreased triglycerides and antioxidant ether phospholipids during the follow-up period were observed in children who developed diabetes (65). Further, increased levels of proinflammatory lysophosphatidylcholines were seen months before seroconversion to autoantibody positivity. Increased odd-chain triglycerides as well as polyunsaturated fatty acid-containing phospholipids and lower concentrations of methionine have also been observed in autoantibody-positive children (66).
2.2 Diabetic complications

2.2.1 Diabetic nephropathy

2.2.1.1 Definition

Diabetic nephropathy is defined as a progressive increase in urinary albumin excretion rate (AER), and a decline in glomerular filtration rate. AER is measured from a timed urine collection (either overnight or 24 h). Microalbuminuria is defined as an increase in AER of \( \geq 20 \) μg/min or \( \geq 30 \) mg/24 h. Macroalbuminuria (also called proteinuria or overt nephropathy) is defined as an increase in AER of \( \geq 200 \) μg/min or \( \geq 300 \) mg/24 h. Several factors can falsely increase AER, e.g. infections, fever, physical exercise, pregnancy, hematuria, menstruation, congestive heart failure, marked hyperglycemia, or hypertension (67). Due to variability in AER, at least two out of three consecutive urine collections are required to define the renal status. Another method for screening is a spot urine sample from which the albumin-to-creatinine ratio (ACR) is measured. The cut-off points for micro- and macroalbuminuria if ACR is used are \( \geq 2.5 \) or \( \geq 3.5 \) mg/mmol and >25 or >35 mg/mmol in men and women, respectively. The final stage of diabetic nephropathy is end-stage renal disease (ESRD), defined as requiring dialysis or a renal transplant.

2.2.1.2 Renal function

Renal function, i.e. glomerular filtration rate (GFR), is classified into five categories by the Kidney Disease: Improving Global Outcomes (KDIGO): stage 1 (normal) = GFR \( \geq 90 \), stage 2 (mildly reduced) = GFR 60-89, stage 3 (moderately reduced) = GFR 30-59, stage 4 (severely reduced) = GFR 15-29, and stage 5 (renal failure) = GFR <15 ml/min/1.73 m\(^2\) (68). GFR can be directly measured by the plasma clearance of inulin or the chromium EDTA method (Cr\(^{51}\)-EDTA) (69, 70). Unfortunately, the direct measurement of GFR is laborious and costly, and therefore, not feasible in the routine clinical setting or in studies with large cohorts. Thus, different mathematical formulas have been developed to calculate the estimated GFR (eGFR). The most often used creatinine-based formulas are the Cockcroft-Gault (71), the Modification of Diet in Renal Disease (MDRD) (72), and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (73) (see Discussion in Section 7.5). Creatinine is a waste product of skeletal muscle, and muscle mass therefore influences creatinine production. It is filtered and mainly secreted by the glomerulus, however, there is also tubular secretion of creatinine. Cystatin C is produced at a constant rate and filtered by the kidneys without active tubular secretion, and it has therefore been suggested as an earlier marker of renal dysfunction than creatinine (74). However, no consensus exists as to whether or not cystatin C should replace creatinine measurements. Equations using both creatinine and cystatin C have also been proposed (75, 76).
2.2.1.3 Epidemiology

Diabetic nephropathy is the key determinant of morbidity and mortality in patients with diabetes (2, 77). It is associated with high CVD risk and is also the most common cause of renal failure in the Western world (12). As diabetes is constantly increasing worldwide, renal failure is also becoming a growing healthcare problem. The peak incidence of nephropathy occurs after a 15- to 20-year duration of diabetes (13). It has previously been shown that around one-third of patients with type 1 diabetes will eventually develop diabetic nephropathy (78), but more recent studies have demonstrated that the incidence of diabetic nephropathy has decreased, probably because treatment of the major risk factors has improved (79).

Microalbuminuria is still the best non-invasive predictor of diabetic nephropathy. In patients with diabetes duration of over 15 years, 28% progressed to macroalbuminuria during a 10-year follow-up (80), whereas in earlier studies up to 80% progressed (81, 82). Regression from microalbuminuria to normal AER is not unusual and has varied from around 30% (13, 80) to as high as 58% in one study (83). Initially, it was thought that in patients with microalbuminuria decline in GFR does not occur (apart from the yearly decline in GFR that results from aging, which is ~1 ml/min/year in individuals over 40 years of age). However, in a cohort from the Joslin Diabetes Center a progressive decline in GFR estimated by cystatin C was observed in 31% of patients with microalbuminuria (84).

After the onset of macroalbuminuria, the average GFR decline is around 10-12 ml/min/year without treatment of hypertension, and hence, the development of ESRD would take around 8-10 years (85, 86). Fortunately, efficient treatment of hypertension will slow down the development of ESRD and even regression from macroalbuminuria to normal AER is possible. In a Finnish study, the cumulative incidence of ESRD was lower than previously reported, 7.8% after 30 years of diabetes duration, and the prognosis of type 1 diabetes was more favorable in patients diagnosed in the more recent years (87).

The increase in AER and the decrease in GFR do not always go hand in hand. In the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) cohort the cumulative incidence of stage 3 chronic kidney disease (CKD, eGFR <60 ml/min/1.73 m²) was 11.4%, and out of these patients 24% were normoalbuminuric, 16% microalbuminuric, and 61% macroalbuminuric (88). The decline in eGFR was 1.2%/year and 5.7%/year in normoalbuminuric and macroalbuminuric patients, respectively.
2.2.1.4 Pathogenesis

Diabetic nephropathy in patients with type 1 diabetes leads to morphological changes in renal arterioles, tubules, interstitium, and, most importantly, the glomerulus. Glomerulopathy, with thickening of the glomerular basement membrane and mesangial expansion, is a hallmark of diabetic nephropathy (89). Glomerular basement membrane thickening occurs first and can be observed as early as 2 years after the onset of diabetes (90). Mesangial expansion, mostly due to an increase in the mesangial matrix, can be observed already after 5-7 years of diabetes onset (91). Diffuse mesangial expansion is associated with the pathognomonic nodular lesions called Kimmelstiel-Wilson nodules (92). Glomerular and tubular basement membrane thickening as well as mesangial expansions are consequences of increased accumulation of extracellular matrix components, e.g. type IV collagen, fibronectin, and laminin (93, 94). Different stages of mesangial expansion and the development of Kimmelstiel-Wilson nodules result in glomerulosclerosis (95). Arteriolar hyalinosis, i.e. exudative lesions in which plasma proteins (e.g. immunoglobulins, fibrinogen, and albumin) may ultimately replace smooth muscle cells, may be present a few years after onset (91, 96). Abnormalities of the glomerular-tubular junction are a late manifestation of the disease (97). In advanced diabetic nephropathy, also tubulointestinal injury, such as inflammation, atrophy, and fibrosis, is observed (98).

It is likely that interactions between several risk factors contribute to the development and progression of diabetic nephropathy. Figure 1 illustrates how hyperglycemia, hypertension, inflammation, and dyslipidemia could lead to the activation of various pathways implicated in the pathogenesis of diabetic nephropathy. Potential pathogenic mechanisms behind lipid-induced renal injury are also discussed in Section 2.3.5.
2.2.1.5 Risk factors

Glycemic control

Long-term glycemic exposure is a prerequisite for the development of diabetic nephropathy, and several studies have demonstrated that poor glycemic control increases the risk of progression of renal disease (99-101). In the Diabetes Control and Complications Trial (DCCT), the development of microalbuminuria was reduced by 39% and progression to macroalbuminuria by 54% in patients with type 1 diabetes with an HbA1c ~7% relative to those with HbA1c ~9% (102). In the post-trial follow-up, the risk of macroalbuminuria was still significantly reduced in the intensively treated group despite similar HbA1c levels after the trial period, suggesting that glycemic memory exists (103). In patients with type 2 diabetes, the United Kingdom Prospective Diabetes Study (UKPDS, HbA1c 7.0% vs. 7.9%), the Action in Diabetes and Vascular Disease (ADVANCE, HbA1c 6.5% vs. 7.3%), and the Veterans Affairs Diabetes Trial (VADT, HbA1c 7.3% vs. 9.3%) have all shown a reduction in microvascular end-points and other beneficial effects of intensified glycemic control (104-106). However, the Action to Control Cardiovascular Risk in Diabetes (ACCORD, HbA1c 6.3% vs. 7.6%) Trial, which
aimed to reduce HbA_1c below 6.0%, had to be discontinued after 3.7 years into the trial due to excess mortality in the intensive treatment group (107). Intensive and rapid decrease of the HbA_1c can lead to increased risk of hypoglycemia and weight gain, and individualization of treatment goals is thus now emphasized. Large glycemic variability has also been shown to predict progression of both micro- and macroalbuminuria (108, 109).

**Blood pressure**

Blood pressure increases in parallel with the increase in AER and is positively correlated with the decline in GFR (110-112). Notably, antihypertensive agents, such as Angiotensin-Converting Enzyme (ACE) inhibitors and Angiotensin II Receptor Blockers (ARBs), have shown renoprotective effects independently of their blood pressure-lowering effects (113, 114). For patients with type 1 diabetes, more evidence of the beneficial effect of ACE inhibitors on diabetic nephropathy prevention is available, and recently a meta-analysis also showed that ACE inhibitors reduce all-cause mortality and major CVD events in patients with diabetes (115), whereas no beneficial effects on these parameters were seen with ARB treatment. Therefore, ACE inhibitors are the first line of treatment for blood pressure reduction in patients with type 1 diabetes. However, most patients need multiple antihypertensive agents to reach the blood pressure treatment targets. Dual blockade with both ACE inhibitors and ARBs has been proposed, but it has raised safety concerns and is not widely recommended. Epidemiological analyses have shown that systolic blood pressure (SBP) >120 mmHg predicts the development of ESRD in the long term, and therefore, a treatment goal of <130/80 mmHg was recommended for patients with diabetes (116). However, recent guidelines have changed the treatment goals back to the less stringent goal of <140/80 mmHg due to lack of evidence of beneficial effects with lower SBP targets (117). In the ACCORD Trial, intensified blood pressure treatment (119 vs. 134 mmHg) reduced albuminuria rates and stroke events, but no beneficial effects were seen on other CVD events or renal function (118). In fact, there was an increase in serious adverse events (e.g. syncope and hyperkalemia). A meta-analysis of 14 randomized clinical trials yielded similar results and concluded that an SBP goal between 130 and 135 mmHg is acceptable (119). These trials included patients with type 2 diabetes with a mean age between 55 and 67 years. An SBP target of <130 mmHg may still be appropriate in younger patients, especially if the target can be achieved with fewer drugs and without side-effects.

**Insulin resistance**

Insulin resistance is not only observed in patients with type 2, but is also a common feature in patients with type 1 diabetes and is observed mainly in the peripheral and hepatic tissues (120). The golden standard for measuring insulin sensitivity is the euglycemic hyperinsulinemic clamp technique (121). Using this technique, insulin resistance was shown to predict the development of microalbuminuria in one study (122), while another found no association between AER and insulin sensitivity (123). Use of the euglycemic hyperinsulinemic clamp is, however, laborious and invasive. Therefore, larger studies have used a surrogate estimate for insulin sensitivity, the estimated glucose
disposal rate (eGDR), which was developed by Williams et al. (124). Notably, in the Pittsburgh Epidemiology of Diabetes Complications (EDC) cohort the eGDR predicted progression to macroalbuminuria (125). Further, in the FinnDiane cohort metabolic syndrome, which is strongly associated with insulin resistance, predicted renal disease progression (126), and having a first-degree relative with type 2 diabetes increased the risk of diabetic nephropathy (127).

Genetics
Diabetic nephropathy clusters in families (128), and in genetically similar patient groups (e.g. Pima Indians, Mexican Americans, Asians, New Zealand Maoris, and Australian Aborigines), the development of diabetic nephropathy is much more common than in individuals of white European origin (129, 130). Therefore, it is likely that genetic factors cause susceptibility to the development of diabetic nephropathy. However, thus far, the results have been a bit disappointing, and very few specific associations between gene variants and diabetic nephropathy have been found. Genes suggested but not shown to be conclusively associated with diabetic nephropathy include e.g. angiotensin-converting enzyme (ACE), engulfment and cell motility 1 (ELMO1), vascular endothelial growth factor (VEGF), apolipoprotein E (APOE), apolipoprotein C-I (APOC-I), and erythropoietin (EPO).

To date the largest genome-wide association study, including the FinnDiane cohort, is the Genetics of Nephropathy - an International Effort (GENIE) Consortium, which identified an intronic single-nucleotide polymorphism (SNP) in the v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4 (ERBB4) gene to be nominally associated with diabetic nephropathy (131). While this association was not of genome-wide significance, the effect was consistent in all cohorts. It is of note that ERBB4 encodes a tyrosine kinase receptor and is involved in tubular development (132).

Smoking
Smoking has been shown to increase the risk of development and progression of diabetic nephropathy in patients with type 1 or type 2 diabetes (133). The pathogenic mechanism is thought to be a deleterious effect of smoking on vascular endothelial cells.

Other risk factors
Other suggested risk factors for diabetic nephropathy are e.g. long diabetes duration (134), male sex (13), anemia (135, 136), low birth weight (137), short adult stature (138), high protein diet (139), adiponectin (140), advanced glycation end-products (AGEs) (141), inflammatory markers such as interleukin-6 (IL-6) and C-reactive protein (CRP) (142), and lipid variables. The role of the lipid profile in the development of diabetic nephropathy is discussed at length in Sections 7.1 and 7.2. Several studies have explored the associations between lipid variables and renal disease (143-146); however, whether the lipid abnormalities precede or occur concomitantly with the increase in AER is still under debate. Furthermore, how retinopathy status affects the association between AER and lipid variables is also unknown.
2.2.2 Diabetic retinopathy

Diabetic retinopathy is a feared complication and the leading cause of adult onset blindness in the working-aged population in the Western world (4). The advanced form of diabetic retinopathy, proliferative diabetic retinopathy (PDR), is sight-threatening and characterized by the proliferation of abnormal new, fragile blood vessels in response to ischemia and vitreous hemorrhage (Figure 2). PDR is preceded by non-proliferative diabetic retinopathy (NPDR), which is classified into different stages depending on the presence and severity of microaneurysms, hemorrhages, retinal edema, lipid exudates, intraretinal microvascular abnormalities (IRMAs), and microinfarcts. A detailed classification of diabetic retinopathy was developed by the Early Treatment of Diabetic Retinopathy Study (ETDRS) (147) in the 1980s and it has become the golden standard to define the severity of diabetic retinopathy.

![Figure 2. Fundus photograph of proliferative diabetic retinopathy with neovascularization (thin arrow) and an occluded artery (thick arrow) as well as scatter laser scars.](image)
Diabetic macular edema involves leakage and exudation at the center of the eye (macula) and may lead to impairment of central vision (Figure 3). Macular edema is more common in elderly patients and therefore also more common in patients with type 2 diabetes. It can be divided into mild (some retinal thickening or hard exudates in the posterior pole, but distant from the center of the macula), moderate (retinal thickening or hard exudates approaching the center of the macula, but not involving the center), and severe (retinal thickening or hard exudates involving the center) (148). Macular edema is generally asymptomatic at early stages, but if it progresses it can cause severe visual disability, especially in older patients (149). It is the most common cause of visual loss in patients with type 2 diabetes (150).

![Figure 3. Fundus photograph of macular edema with hard exudates (=lipid breakdown products due to vascular leakage=thin arrow) and intraretinal hemorrhages (thick arrow).](image)

### 2.2.2.1 Epidemiology

With a sufficiently long duration of diabetes, nearly all patients with type 1 diabetes will eventually have some degree of diabetic retinopathy (15, 151), however, the rate of the progression and the severity of retinopathy varies substantially. The most profound increase in the incidence of PDR is usually seen after a 10 year duration of diabetes (152).
A number of studies have looked at the prevalence and incidence of diabetic retinopathy, but the results have been very conflicting due to differences in methodology (153). In the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), a population-based study including 995 patients with type 1 diabetes, the 25-year cumulative incidence of any retinopathy was 97%, and the incidence of PDR was 42% (15). Due to improved management of diabetes and related risk factors, a reduction in the incidence of retinopathy has been noted in recent studies. In a meta-analysis including patients with diabetes without ocular treatment for retinopathy at baseline, the 4-year incidence of PDR in studies performed before and after 1985 was 19.5% and 2.6%, respectively (154). Also in the FinnDiane cohort, a reduction in the cumulative incidence of severe diabetic retinopathy over the last decades has been observed (155).

Prompt laser treatment is crucial for the prevention of severe vision loss in patients with PDR (156). However, laser treatment destroys retinal tissue, and the desired result is not always achieved, and sometimes treatment is initiated too late. Therefore, comprehensive screening and treatment and recognition of risk factors are crucial for the prevention of diabetic retinopathy.

2.2.2.2 Risk factors

Hyperglycemia is the most important modifiable risk factor for prevention of diabetic retinopathy. Strong evidence indicates an association between poor glycemic control and worsening of diabetic retinopathy and between improved glycemic control and favorable outcome. The first long-term study conducted for the period 1947-1973 followed 4398 patients with diabetes and showed that poor glycemic control assessed cumulatively over the years is related to a higher incidence and progression of retinopathy (157). In the DCCT Study, intensified glycemic control in patients with type 1 diabetes for a mean of 6.5 years resulted in a 47% reduction of the risk of severe NPDR or PDR (158). Further, intensified treatment reduced the incidence of retinopathy by 76% and slowed down the progression of retinopathy by 54% in patients with retinopathy at baseline. With HbA1c above 6.5%, there is a clear increase in the prevalence of retinopathy, which partly also explains why this threshold has been chosen for the diagnosis of diabetes (23). Paradoxically, a transient worsening of diabetic retinopathy is seen if the glycemic control improves too rapidly, a phenomenon called “early worsening” (159, 160), and similarly as for the diabetic nephropathy progression, large variability of the HbA1c was a risk factor for diabetic retinopathy in both the DCCT (108) and the FinnDiane cohort (161).

Elevated blood pressure is associated with the progression of diabetic retinopathy in patients with type 1 diabetes (162), and antihypertensive treatment is associated with slower progression of diabetic retinopathy. In the UKPDS Trial, including hypertensive patients with type 2 diabetes, tighter blood pressure control (defined as <150/85 mmHg) resulted in a 35% reduction in the need for laser treatment and 25% less patients with a ≥2-step progression on the ETDRS severity scale (163). In the EURODIAB Study,
treatment with an ACE inhibitor, lisinopril, in patients with type 1 diabetes reduced diabetic retinopathy progression by 50% after only 2 years of follow-up (164). Further, treatment with an ARB, candesartan, also reduced the incidence of diabetic retinopathy in patients with type 1 diabetes (165) and increased regression of diabetic retinopathy in patients with type 2 diabetes (166). However, no additional benefits from intensively lowered blood pressure targets (SBP <120 mmHg) have been found (167).

Duration of diabetes is the strongest non-modifiable risk factor for diabetic retinopathy. After a 5-year duration of diabetes, the prevalence of diabetic retinopathy is only 17%, whereas in those with ≥15 years it is as high as 97.5% (168).

Pregnancy causes a transient increase in the risk of diabetic retinopathy, but fortunately the long-term risk of diabetic retinopathy seems to be unaffected (169, 170).

Smoking has also been associated with the progression of diabetic retinopathy, however, the results have been somewhat inconsistent and suggested to be mediated through the poorer glycemic control observed in smokers (171, 172).

Other risk factors associated with retinopathy are e.g. anemia (173), waist-hip ratio (WHR) (174), recent cataract surgery (175), puberty (176), and heavy alcohol consumption (177). Dyslipidemia has also been suggested to be a risk factor for diabetic retinopathy, but previous studies have yielded conflicting results (17-20). In fact, most studies to date have reported a lack of an association between lipid variables and diabetic retinopathy (178), leading to the conclusion that traditional lipid variables are most likely not related to diabetic retinopathy.

Diabetic nephropathy and retinopathy are strongly associated with each other and share several risk factors and mechanisms of disease progression (16). Micro- and macroalbuminuria are strongly associated with diabetic retinopathy, especially in patients with younger age at onset (179). However, renal status has been unknown or not taken into account in most retinopathy studies, and thus, comparison between studies is problematic when the prevalence of renal disease has most probably varied between the studied cohorts. The interactions between diabetic retinopathy, nephropathy, and lipid variables have also not been studied previously. How these interactions affect the associations between complications and lipid variables is thus unknown.

2.2.3 Diabetic neuropathy

Diabetic neuropathy is the most common form of neuropathy in industrialized countries, and in clinical practice the simple definition of “the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes” (180) can be used. The prevalence of diabetic nephropathy increases with longer diabetes duration and has been reported to be around 50% after 15-20 years of diabetes (181),
although large variation is present in the prevalence estimates between studies. Screening for neuropathy is recommended 5 years after the onset of type 1 diabetes, after which an annual examination is advised (117). Distal symmetric polyneuropathy (DPN) is the most common form of diabetic nephropathy (182), and clinical tests for its detection include vibration sensation assessment, monofilament perception, and ankle reflex testing. Around one-third of all patients with diabetic neuropathy suffer from unpleasant sensory symptoms such as pain, numbness, burning sensation, or tingling (183). A wide range of medications, e.g. pregabalin, venlafaxine, duloxetine, gabapentin, amitriptyline, valproate, and opioids is used to treat neuropathic pain (184), which is often, however, resistant to treatment. No specific treatment for the underlying nerve damage is available, except improved glycemic control, which has been shown to prevent the development and progression of the disease (185). The symptoms can also be diminished by avoidance of extreme fluctuations in blood glucose levels (117). Up to half of the patients with DPN may be asymptomatic, however, due to loss of sensation they have an increased risk of foot injury. Foot ulcers are often initiated by diabetic neuropathy and together with peripheral arterial disease, neuropathy is the major underlying cause of diabetic foot ulcers and amputation of lower extremities, which cause suffering and disability and are laborious and costly for the healthcare system (186).

Diabetic autonomic neuropathy can cause e.g. exercise intolerance, orthostatic hypotension, gastroparesis, constipation, diarrhea, erectile dysfunction, loss of bladder control, silent ischemia, impaired sweat gland innervation, and decreased response to hypoglycemia. Cardiovascular autonomic neuropathy (CAN) can be detected by changes in heart rate variability and an abnormal response to deep breathing, standing, and the Valsalva maneuver. An advanced stage of CAN is indicated by resting tachycardia (pulse >100/min) and orthostatic hypotension (i.e. a decrease in SBP [20 mmHg] or diastolic blood pressure [DBP, 10 mmHg] after standing up without an appropriate heart rate response) (117). CAN is an independent risk factor for cardiovascular mortality (187), and a multifactorial intervention targeting hyperglycemia, hypertension, smoking, dyslipidemia, and other lifestyle risk factors has been shown to reduce the development and progression of autonomic neuropathy in patients with type 2 diabetes (188).

2.2.4 Macrovascular complications

Cardiovascular disease (CVD) is the major cause of death in patients with type 1 diabetes (21). The main clinical features of CVD are coronary artery disease (CAD), stroke, and peripheral vascular disease. The risk of cardiovascular morbidity and mortality is at least 2- to 4-fold higher in patients with type 2 diabetes than in the general population (189, 190). The risk has previously been considered to be of similar magnitude in patients with type 1 diabetes, but recent studies have shown that the additional mortality risk is strongly connected to the renal status. In the FinnDiane cohort, patients with type 1 diabetes with a normal AER showed no excess in mortality beyond that of the general population, whereas microalbuminurina was associated with a 3-fold, macroalbuminurina with a 9-fold,
and ESRD with an 18-fold increase in all-cause mortality (2). The results have later been replicated in the Pittsburgh EDC cohort with a 20-year follow-up period (3). The reason for the strong association between CVD and diabetic nephropathy remains unknown. The two complications share several risk factors and are thought to develop in parallel, but it can also be argued that CVD develops as a consequence of the hypertension, inflammation, and dyslipidemia caused by diabetic nephropathy.

The most well-known risk factors for both CVD and diabetic nephropathy are microalbuminuria, decrease in GFR, age, duration of diabetes, hypertension, glycemic control, obesity, insulin resistance, smoking, genetic predisposition, and dyslipidemia (130). The reports on hyperglycemia as a risk factor for CVD have been conflicting, and glycemic control may be more important for microvascular than macrovascular complications. However, glycemic control predicts coronary artery calcification (CAC), and in the DCCT/EDIC Study, intensive glycemic treatment was associated with less atherosclerosis in the period after the original trial (191, 192). Moreover, in a Finnish study, an increment in HbA1c of 1% increased CVD mortality by 52.5% (95% confidence interval [CI] 28.4–81.3) in patients with type 1 diabetes and by 7.5% (4.3–10.8) in patients with type 2 diabetes (193).

2.2.4.1 Coronary artery disease

Type 1 diabetes is associated with earlier onset and faster progression of atherosclerosis (1, 194). Carotid intima-media thickness, which is considered a surrogate marker for early atherosclerosis, is increased in patients with type 1 diabetes and is similar to the levels of healthy control subjects who are 20 years older (195). Further, in an earlier study, 35% of patients with type 1 diabetes died of CAD by age 55, compared with only 8% and 4% of non-diabetic men and women, respectively (196). In a more recent study, the standardized mortality rates from CAD were 9 times higher in men and 42 times higher in women with type 1 diabetes under the age of 40 years (197). The protective effect of female sex is lost in women with type 1 diabetes, which leads to the higher standardized mortality rates observed in women in several studies (193, 196, 197). Type 1 diabetes is also associated with a higher frequency of asymptomatic CAD and as many as 24% of asymptomatic patients with type 1 diabetes between 35 and 60 years of age have ischemia on either exercise electrocardiography, 24-h Holter monitoring, or dynamic perfusion scintigraphy (198). CAD in patients with type 1 diabetes is more diffuse, with a higher likelihood of stenosis of all three major coronary arteries in the distal segments (199). The involvement of distal segments renders the patients frequently unsuitable for bypass grafts. The mortality rate of CAD events in patients with diabetes is also higher (200, 201).

Despite lipid variables and CAD events being extensively studied in the general population and in patients with type 2 diabetes, data on patients with type 1 diabetes are surprisingly scarce. Which lipid variable is the best predictor of an incident CAD event and how concomitant renal status affects this relationship are unknown.
2.3 Lipoprotein metabolism

Lipoprotein particles transport non-water-soluble cholesterol and triglycerides in plasma and consist of a hydrophilic surface monolayer of phospholipids, free cholesterol, and apolipoproteins (Apo) and a central core of hydrophobic cholesterol esters and triglycerides. They can be classified into five major classes according to their hydrated density by ultracentrifugation: chylomicrons (density (d) < 0.93 g/ml), very-low-density lipoproteins (VLDL, d=0.93-1.006 g/ml), intermediate-density lipoproteins (IDL, d=1.006–1.019 g/ml), low-density lipoproteins (LDL, d=1.019–1.063 mg/dl), and high-density lipoproteins (HDL, d=1.063–1.21 mg/dl) (202).

The largest lipoprotein particles, chylomicrons, are responsible for the transport of dietary triglycerides and cholesterol. They consist mainly of triglycerides (~85-90%), cholesterol esters, phospholipids, and ApoB₄₈. ApoB₄₈ is intestinally produced and has 48% of the molecular weight of ApoB₁₀₀ (hereafter referred to as ApoB). In addition to ApoB₁₈, chylomicrons also contain ApoA-I and acquire ApoC-I, -II, -III, and ApoE from HDL particles. The close interrelationship between the metabolic pathways for HDL particles and triglyceride-rich lipoproteins is illustrated in Figure 4. In the circulation, the triglycerides of the chylomicrons are hydrolyzed by the enzyme lipoprotein lipase (LPL) into triglyceride-poorer particles, i.e. chylomicron remnants, which are taken up by the liver.

VLDL particles are secreted by the liver and compromise endogenous triglycerides (~55%) cholesterol (~25%), phospholipids (~18%), and ApoB, ApoA-II, ApoC-I, -II, -III and ApoE (203). In the circulation, the triglycerides of the VLDL are also hydrolyzed by LPL. During this process phospholipids, ApoC, and ApoE are transferred to HDL particles and the VLDL particles are in turn transformed into IDL particles.

IDL particles contain ApoB and ApoE and lie between the VLDL and LDL particles in their composition (203). IDL particles can be taken up by the liver or they can be further metabolized to LDL particles by the enzyme hepatic lipase (HL).

LDL particles consist of triglycerides (~6%), cholesterol (~55%), phospholipids (~20%), and ApoB and are the main cholesterol-bearing lipoproteins in the plasma (203). Each VLDL-IDL-LDL particle contains only one ApoB molecule, and therefore measuring ApoB works as a marker of the number of these atherogenic lipoprotein particles in the circulation (204). As the plasma residence time of VLDL is ~2-6 h, IDL ~1 h, and LDL ~1.5-3 days (203, 205), about 90% of the circulating ApoB is found in the LDL particles (206).

Lipoprotein(a) (Lp[a]) is an LDL-like lipoprotein, but in addition to ApoB, the glycoprotein apolipoprotein(a) is also attached to the particle. Most people have very low concentrations of Lp(a), but 2- to 4-fold higher concentrations are found in people of African origin (207, 208). Most methods to measure or calculate LDL cholesterol
concentrations do not distinguish between cholesterol derived from LDL or Lp(a), and therefore, the reported LDL cholesterol is the net sum of cholesterol from both lipoprotein particles.

HDL particles are secreted by the liver as small, lipid-poor lipoproteins, containing mostly ApoA-I. HDL particles are in a constant state of lipidation and delipidation and remodeling. ApoA-I constitutes about 70% of the protein content of HDL particles (209), and each HDL particle contains one to five copies of ApoA-I. In addition, ApoA-II, -IV, -V, ApoC-I, -II, -III, and ApoE may be present. All in all, HDL particles compromise over 100 proteins, which are considered to play an important role for the function of the HDL particles (203). Nascent HDL particles receive cholesterol and phospholipids from peripheral cells via the ATP-binding cassette A1 transporter. Within the HDL particles, free cholesterol is esterified by the enzyme lecithin–cholesterol acyltransferase (LCAT), leading to the formation of HDL₃ particles. Thereafter, the phospholipid transfer protein (PLTP) enzyme promotes the fusion of two denser HDL₃ particles, leading to the formation of one, more buoyant HDL₂ particle (210). HDL₂ particles are degraded by HL and endothelial lipase to HDL remnant particles, which are taken up by the liver by the scavenger receptor (211).

Figure 4. Metabolic pathways for triglyceride-rich lipoprotein remnants and HDL particles (modified from Chapman MJ et al., European Heart Journal 2011) (212).
2.3.1 Actions of insulin on lipoprotein metabolism

By inhibiting the HL, insulin enhances the storage of triglycerides in the adipose tissue as well as reduces the release of free fatty acids from the adipose tissue. Insulin has multiple sites of action on lipid metabolism in the liver (Figure 5). It inhibits VLDL production in the liver (213) and activates LPL in adipocytes (214), which promotes the catabolism of triglyceride-rich lipoproteins (TRLs) (i.e. chylomicrons and VLDL particles). Moreover, insulin decreases ApoB secretion by promoting ApoB degradation in the liver (215) and also enhances the clearance of LDL by increasing the LDL B/E receptor activity (216).

Figure 5. VLDL secretion from the liver is regulated by insulin through several pathways (green arrows) and is increased in insulin-resistant states (red arrows) (modified from Choi SH and Ginsberg HN, Trends in Endocrinology and Metabolism 2011) (217). Insulin suppresses FA oxidation and de novo lipogenesis. It also suppresses MTP synthesis, which is a rate-limiting step in hepatic VLDL production. Insulin can also directly affect apolipoprotein (Apo) B secretion by targeting it for degradation, which inhibits VLDL secretion. Expression of ApoC-III, an inhibitor of lipoprotein lipase, is also suppressed by insulin. In insulin-resistant states, there is an increase in the hepatic secretion of VLDL particles due to increased hepatic triglycerides from the enhanced fatty acid flux to the liver, the excess availability of ApoB, and the increased de novo lipogenesis.

CM = chylomicron, FFA = free fatty acids, FA = fatty acids, DNL = de novo lipogenesis, TG = triglycerides.

In patients with newly diagnosed type 1 diabetes with ketoacidosis and insulin deficiency, a reduction of triglyceride-rich lipoprotein catabolism and a profound increase in TRLs, mainly because of decreased LPL activity, can be observed (218, 219). HDL cholesterol concentrations are also significantly decreased as a consequence of hypertriglyceridemia (220). In contrast, in patients with type 1 diabetes and good glycemic control, the triglyceride concentration is usually normal or slightly decreased (218, 219, 221), due to enhanced downregulation of VLDL production by increased plasma insulin concentrations.
as a consequence of subcutaneous insulin treatment (218, 222). Further, patients with type 1 diabetes display peripheral hyperinsulinemia, which increases the activity of LPL, thereby lowering triglyceride concentrations (223). Plasma LDL cholesterol is normal or even slightly decreased in patients with intensified insulin treatment as a consequence of decreased VLDL production (223, 224). HDL cholesterol concentrations are normal or slightly increased in patients with good glycemic control (219). The increase in HDL cholesterol may be due to an increase in the LPL/HL ratio (due to increased LPL activity and normal HL activity) (225), again as a consequence of peripheral hyperinsulinemia caused by subcutaneous insulin treatment. Implantable insulin pumps with an intraperitoneal insulin administration route mimic the physiological route of insulin and should not lead to the peripheral hyperinsulinemia and hepatic hypoinsulinemia that the subcutaneous route does. Studies evaluating the modification of the lipid profile after the replacement of subcutaneous with intraperitoneal insulin treatment have, however, yielded conflicting results. The HDL cholesterol concentrations have been shown to be decreased (226) or unchanged (227-229), the triglycerides increased (226) or unchanged (227-229), and the total cholesterol and ApoB unchanged (227, 228). Even though the subcutaneous route of insulin administration occasionally seems to be associated with more favorable quantitative changes in the lipid profile, it could, however, also be associated with unfavorable qualitative changes, which could affect the function of the lipoprotein particles.

2.3.2 Insulin resistance and lipoprotein metabolism

Insulin resistance is a typical feature of the metabolic syndrome and type 2 diabetes, but as the prevalence of overweight and obesity is increasing in the society this feature is becoming more common in patients with type 1 diabetes as well. Intensive glycemic control can also cause overweight and insulin resistance, and these features are especially common in those with a family history of type 2 diabetes. Moreover, renal disease is associated with insulin resistance already at the early stages in patients both with and without diabetes (230, 231). Insulin resistance is also considered to be a strong pathogenic contributor to the progression of renal disease (232, 233).

Because LPL is insulin-dependent, its activity is commonly reduced in patients with insulin resistance (234), resulting in longer residence times of the TRLs in the circulation. The increased amounts of VLDL particles result, in turn, in an increased triglyceride content in LDL particles through the action of the cholesteryl ester transfer protein (CETP) (235). Triglyceride-rich LDL particles are good substrates for HL, which hydrolyzes triglycerides, making the LDL particles smaller and denser (236). Small, dense LDL (sdLDL) particles are frequently present in insulin-resistant states (237). In children with type 1 diabetes, as many as 87% had a phenotype dominated by the presence of sdLDL compared with only 11% in children without diabetes (238). SdLDL particles have been found to be more atherogenic than the large, buoyant LDL particles for several reasons: i) hepatic LDL receptors have a lower affinity for sdLDL particles, which leads to
a prolonged plasma retention time of these particles (239), ii) sdLDL particles show increased binding to intimal proteoglycans, which could favor penetration into the arterial wall (240), iii) they are more effective in promoting lipid accumulation in macrophages, which leads to an increased formation of foam cells (241), iv) they are more likely to undergo glycation (242), and v) they are more susceptible to oxidation (243, 244). Oxidized LDL (oxLDL) particles are rapidly taken up by macrophages and promote the formation of cytokines (e.g. tumor necrosis factor alpha [TNF-α] and IL-6) by macrophages, accelerating the inflammatory atherosclerotic process (245). In insulin-resistant states, the LDL cholesterol concentrations are only slightly increased or similar to those of controls, but the number of LDL particles is increased (246) (see Figure 6 for illustration). Thus, as 90% of the circulating ApoB is found in LDL, ApoB is consequently also increased when insulin resistance is present and can be seen as a surrogate marker for the number of LDL particles (246).

![Large, buoyant LDL](image1.png) ![Small, dense LDL](image2.png)

**Figure 6.** Illustration of the same LDL cholesterol concentrations with either normal or elevated apolipoprotein (Apo) B concentrations.

The main reason for the decreased HDL cholesterol concentrations in insulin resistance seems to be the increased transfer of triglycerides from the TRLs to the HDL particles and the reciprocal transfer of cholesterol from the HDL particles to the TRLs via the CETP enzyme (235). Triglyceride-rich HDL particles are also good substrates for HL, making them smaller and denser, and this increases the catabolism and clearance of HDL particles from the plasma (247). The reduced HDL concentrations in insulin-resistant states are typically seen as reduced HDL$_{2b}$ subspecies and an increase in the smaller and denser HDL$_{3b}$ and HDL$_{3c}$ subspecies (248). Mechanisms affecting HDL particles in insulin resistance are shown in Figure 7.
2.3.3 Apolipoproteins in diabetes

Insulin exerts multiple effects on the major apolipoproteins (ApoB, ApoA-I, ApoC-III, and ApoE). In patients with type 1 diabetes and fairly good glycemic control, the ApoB concentrations are within the normal range (224) and the ApoA-I concentrations are slightly elevated or within the normal range (250). In normal physiology, insulin promotes ApoB degradation in the hepatocyte and leads to decreased ApoB secretion from the liver (215). In type 1 diabetic patients with ketoacidosis and total lack of insulin, the ApoB concentrations are in the upper normal range, but decrease during insulin treatment with a concordant and significant decrease in VLDL ApoB, but not in IDL or LDL ApoB (220). In insulin-resistant states, the ApoB degradation is decreased, there is an increased production of large VLDL\(_1\) particles, and the catabolic rate of ApoB-containing lipoproteins, especially IDL and LDL, is reduced (215, 251). Interestingly, increased ApoB concentrations can already be observed in children with type 1 diabetes as well as in healthy children with diabetic parents compared with healthy children with non-diabetic parents (252). The ApoA-I concentrations are near the normal range already before the initiation of insulin treatment in type 1 diabetic patients with ketoacidosis; however, the ratio of ApoA-I to cholesterol in the HDL particles falls during treatment (220). In insulin-resistant states, the triglyceride loading of core HDL leads to rapid triglyceride lipolysis and the formation of denser HDL particles, and the loss of HDL core triglycerides, in turn, leads to the release of ApoA-I from HDL particles. ApoA-I then undergoes glomerular filtration and the catabolic loss of ApoA-I is increased by 48% (251, 253). However, ApoA-I production is increased by 25%, probably due to a compensatory mechanism, but the net effect is still a reduction in ApoA-I concentrations.
In insulin-resistant states, the inhibitory role of insulin in ApoC-III expression may be lost and the high glucose levels may further stimulate ApoC-III expression (254). Increased free fatty acid delivery to the liver also increases the ApoC-III secretion. ApoC-III inhibits the LPL-mediated catabolism of VLDL and the uptake of VLDL by the liver and may also increase VLDL secretion (203). The ApoE polymorphism influences total and LDL cholesterol as well as ApoB concentrations, but the allele frequency of ApoE in patients with type 1 diabetes does not differ from that of the general population in Finland (218). Glycation is also likely to affect the function of apolipoproteins, and in patients with hyperglycemia glycation of ApoA-I, ApoA-II, ApoB, ApoC-I, and ApoE has been observed (255).

2.3.4 Secondary changes in lipid profile in nephrotic syndrome

Nephrotic syndrome is defined as proteinuria of >3.5 grams/24 h/1.73 m² (256). It is accompanied by hypalbuminemia, edema, thrombophilia, increased risk of infections, and dyslipidemia. The low plasma albumin concentrations, the oncotic pressure, and the renal protein leakage are thought to play important roles in the development of lipid abnormalities. Impaired catabolism and increased synthesis of ApoB-containing lipoproteins and their remnants as well as an accumulation of oxLDL particles is observed in patients with the nephrotic syndrome (257, 258). Lp(a) has also been found to be elevated in patients with the nephrotic syndrome (259), but this can be reversed by antiproteinuric treatment (260). The plasma concentrations of total HDL cholesterol are often normal in patients with nephrotic syndrome, but the maturation of the HDL particles is impaired and qualitative alterations can therefore be seen. Urinary loss of LCAT results in plasma LCAT deficiency and as a consequence the maturation of the small, dense HDL₃ particles to large, buoyant HDL₂ particles is altered (261). This leads to high concentrations of HDL₃ and low concentrations of HDL₂ particles and may also cause disturbances in the reverse cholesterol transport system.

2.3.5 Possible mechanisms for lipid-induced renal injury

The mechanisms by which dyslipidemia could cause or induce renal injury remains unclear, but similarities between atherosclerosis and glomerulosclerosis were recognized already more than 20 years ago (262). Notably, genetic lipid disorders, such as deficiency in LCAT, abnormalities in ApoE, and familial type III hyperlipoproteinemia, lead to renal disease (263-265). Further, when guinea-pigs and rats are fed cholesterol-rich food, they develop various forms of glomerular and other injuries (266, 267). On the other hand, lipid abnormalities alone may be insufficient to cause renal injury since dyslipidemia in non-diabetic human individuals is rarely associated with renal disease. Therefore, there is likely a trigger that causes the initial renal injury, which is then aggregated by dyslipidemia. Hyperglycemia, hypertension, or inflammation, often seen in patients with diabetes, could serve as such a trigger (see also Figure 1).
Lipoproteins enhance matrix expansion, mesangial cell proliferation, and mesangial cytokine production (268-270). The increased cytokine production may recruit macrophages, and similarly as observed in the arterial wall, infiltration of macrophages and foam cells can be found in the glomeruli of patients with diabetic nephropathy (271). Hyperglycemia causes mitochondrial overproduction of reactive oxygen species (ROS) and synthesis of AGEs (272). Further, glycated VLDL and LDL particles are more susceptible to oxidation (273), and AGE-modified LDL particles are cleared from the plasma more slowly (274). As the kidney is a major site for AGE adduct clearance, it is thought that AGE-modified lipoproteins may damage the glomeruli. Also, hyperlipidemia on its own can promote inflammation and the generation of ROS by monocytes (275). Both monocytes and mesangial cells may oxidize lipoproteins (275, 276), and oxLDL particles may serve as chemoattractants for both T-lymphocytes and macrophages (277). Scavenger receptors on the mesangial cells have higher affinity for oxLDL than for native LDL, and stimulation by oxidized lipoproteins leads to mesangial cytokine production and further recruitment of monocytes (274). Cytokine production by tubular epithelial cells is stimulated by the presence of cytokines and high molecular proteins in the glomerular filtrate. Further, oxLDL particles may increase apoptosis of mesangial cells, endothelial cells, and podocytes (278-280). OxLDL can also cause vasoconstriction by increasing the production of vasoactive substances and reducing the production of vasodilators (281). Inflammatory factors, such as TNF-α, ROS, and oxLDL, may cause disruption of the glycocalyx, which is vasoprotective and influences the glomerular permeability (282). OxLDL, hyperglycemia, and ROS can also stimulate the production and activity of the transforming growth factor (TGF)-β (274, 283). TGF-β activation leads to an increase in the synthesis of extracellular matrix proteins and an impairment in extracellular matrix degradation (284). Moreover, TGF-β stimulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 2 (Nox2), and Nox4 expression in kidney fibroblasts, activating the extracellular signal-regulated kinase ½ (ERK½) pathway. This results in conversion of fibroblasts to a myofibroblast phenotype, which is associated with interstitial fibrosis (285). Dyslipidemia may also cause alterations in the coagulation-fibrinolysis system, a decreased renal blood flow, and endothelial cell damage (286).

2.4 Lipid-lowering treatment

Statins inhibit the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme, which plays a central role in hepatic cholesterol synthesis (287). Strong evidence indicates that statins reduce CVD events in patients with diabetes, but almost all trials in patients with diabetes have mainly included patients with type 2 diabetes. The trials including the largest numbers of patients with diabetes are the Heart Protection Study (HPS) (288), the Anglo-Scandinavian Cardiac Outcomes Trial – Lipid-Lowering Arm (ASCOT-LLA) (289), the Collaborative Atorvastatin Diabetes Study (CARDS) (290), and the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial – Lipid-Lowering Trial (ALLHAT-LLT) (291) including almost 15 000 patients with
diabetes. The largest cohort of patients with type 1 diabetes is found in the HPS with 615 patients (288). In this particular trial, the magnitude of the reduction in CVD events was similar in patients with type 1 and type 2 diabetes, although the reduction was not statistically significant in the former group due to lower power. In a meta-analysis of 14 trials including 18,686 patients with diabetes (1466 with type 1 diabetes), major vascular events were reduced by 21% per 1 mmol/l reduction in LDL cholesterol during an average follow-up of 4.3 years (292). The findings were independent of the baseline lipoprotein concentrations, and similar benefits were seen irrespective of age, sex, type of diabetes, or kidney status. Based on this meta-analysis, the number needed to treat (NNT) to prevent one vascular event was as low as 9 for high-potency statins and 22 for low-potency statins. Economic analyses of randomized statin trials, including the HPS, have shown that statin treatment is cost-effective for a wide range of patients with diabetes (293).

The lipid-lowering mechanisms of the fibrates, i.e. peroxisome proliferation activator receptor (PPAR)-α agonists, include activation of LPL and reduced production of ApoC-III, leading to an increased clearance of VLDL and IDL particles (294). Rather inconsistent results have been reported on the effect of fibrates on cardiovascular outcomes. In a meta-analysis with 45,058 participants, a 13% relative risk (RR) reduction was found for CAD events, but no benefits on stroke or all-cause mortality were seen. In patients with type 2 diabetes, the results regarding the overall primary end-points have been disappointing, but beneficial effects on CVD risk in patients with dyslipidemia (defined as high triglycerides and low HDL cholesterol) at baseline have consistently been reported (295-297). In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study, a significant 27% RR reduction was observed in patients with marked dyslipidemia (triglycerides ≥2.3 mmol/l alone or with low HDL cholesterol concentrations) at baseline treated with fenofibrate for 5 years compared with the non-significant 11% RR reduction in the entire cohort (296).

2.4.1 Lipid-lowering treatment and progression of renal disease

Randomized clinical trials of lipid-lowering treatment with data on renal disease progression are listed in Table 2.

Treatment with pravastatin or simvastatin has been associated with a modest reduction in the rate of eGFR decline in non-diabetic cohorts with (or with a high risk of) coronary heart disease (298, 299), and instead of the expected 5-year decline in eGFR, an improvement of eGFR was seen in patients with atorvastatin treatment (300). In the HPS trial, including patients with diabetes or occlusive arterial disease, simvastatin treatment was associated with a smaller decrease in eGFR, and the effect was slightly larger among patients with diabetes (288). In the CARDS Trial, comprising patients with type 2 diabetes without prior CVD, atorvastatin treatment was associated with a modest improvement in the annual change in eGFR (301), and in a substudy of the Treating to New Targets (TNT) trial in patients with diabetes and CAD, 10 mg and 80 mg of atorvastatin increased eGFR
in patients with or without moderate CKD, with a more significant increase in eGFR in patients treated with the higher atorvastatin dose (302). In a meta-analysis of 39,704 patients, including both patients with and without diabetes, the eGFR decline was 1.22 ml/min/year slower with statin treatment; however, subgroup analyses showed no significant differences in patients with diabetic nephropathy and the between-study heterogeneity was considerable (303). In a recently published meta-analysis of 41 trials with a total of 88,523 patients, statin treatment modestly reduced the rate of decline in eGFR compared with placebo (standardized mean difference 0.15, p=0.0004) (304). Twenty-one of these trials with a total of 3933 patients reported data on urinary protein excretion, and a modest decrease in proteinuria with statin treatment was also seen. High- and moderate-intensity statins significantly decreased the rate of reduction of eGFR, whereas the difference between low-intensity statins and placebo was non-significant. The authors concluded that the beneficial effect of statin treatment might be dosage-related and duration-dependent. Statins reduced the decline in eGFR in patients with stage 1 to 3 CKD, but data were insufficient to analyze the effect of statins on patients with stage 4 to 5 CKD.

In the Justification for the Use of Statins in Prevention - an Intervention Trial Evaluating Rosuvastatin (JUPITER), no effect on eGFR was seen after 12 months of rosvastatin treatment (305), and a combination therapy of simvastatin and ezemibib (an inhibitor of cholesterol absorption in the gut) did not affect measures of kidney disease in pre-dialysis patients in the Study of Heart and Renal Protection (SHARP) Trial (306). Further, fluvastatin treatment had no effect on the incidence of renal graft loss, doubling of serum creatinine, or decline in GFR during a 5-year follow-up of 2102 renal transplant recipients in the Assessment of Lescol in Renal Transplantation (ALERT) Study (307, 308). However, in a meta-analysis including nine trials with atorvastatin treatment for 4194 patients with pre-dialysis CKD, a significant effect on eGFR was reported (309).

Fenofibrate treatment is associated with an initial increase in plasma creatinine, and hence, a reduction of eGFR, but the increase has been reported to be reversible (310, 311), and in a small study a reduction of GFR assessed by inulin clearance was not observed (312). The results from the FIELD Study have been conflicting. In the FIELD Helsinki Substudy, a decrease in eGFR and an increase in cystatin C with fenofibrate treatment were reported (313). In another substudy of the FIELD cohort, fenofibrate initially decreased eGFR, but after a washout period, eGFR had fallen less from baseline with fenofibrate than with placebo treatment, 1.9 vs. 6.9 ml/min/1.73 m² (p<0.001, after ~5 years), and a greater benefit of eGFR preservation was seen with fenofibrate treatment in those with baseline dyslipidemia (i.e. high triglycerides and low HDL cholesterol) (310). In the Diabetes Atherosclerosis Intervention Study (DAIS), fenofibrate treatment reduced progression to microalbuminuria, but the mean values of AER did not change (314). In the main results of the FIELD Study, fenofibrate treatment modestly reduced the pooled end-point of progression or regression of albuminuria status (295); however, in the Helsinki Substudy, no beneficial effect on AER analyzed as a continuous variable could be seen (313). The results of the FIELD Study may have been weakened by the use of other lipid-lowering
treatment (mainly statins) in 36% of patients in the placebo group and in 19% of patients in the fenofibrate group by the end of the study (295). However, in the ACCORD Study, all patients received simvastatin treatment, but on top of this patients were randomized to receive either fenofibrate or placebo (297). The combination of fenofibrate and simvastatin modestly reduced progression to micro- or macroalbuminuria compared with simvastatin treatment alone. In a meta-analysis with 14 385 patients with type 2 diabetes, fenofibrate reduced the risk of albuminuria progression by 14% (315). Data on regression of albuminuria status were available for 2152 patients, and the likelihood of regression increased (RR 1.19) with fenofibrate treatment.

All in all, lipid-lowering treatment seems to have a modest beneficial effect on the decline of eGFR and the development and progression of albuminuria. Most of the current evidence on lipid-lowering treatment comes from patients with vascular disease already present at baseline, and it is not known whether lipid-lowering intervention at an earlier stage could provide benefits that may be lost at the later stages of diabetic nephropathy. Further, the follow-up times of most randomized clinical trials are fairly short, and larger benefits would likely be observed with longer treatment periods initiated earlier in the course of the disease. For example, a loss-of-function mutation leading to a lifelong reduction of LDL cholesterol of ~1.0 mmol/l was associated with ~88% reduction of CAD (316), whereas LDL cholesterol lowering of a similar magnitude with statin treatment for 5 years reduces CAD events by ~35% (317). Animal studies have also suggested that the combined effect of ACE inhibitors and statins might provide larger renal benefits than either drug alone (318). In the future, the Adolescent type 1 Diabetes, cardio-renal Intervention Trial (AdDIT) will show the results of the combined effect of an ACE inhibitor (quinapril) and atorvastatin on early surrogate measurements of diabetic nephropathy and CVD (319) and hopefully provide us with new insight. Furthermore, trials with hard renal end-points and direct GFR measurements (not only eGFR, which is dependent on creatinine production and excretion) are also needed to clarify the situation.
Table 2. Studies of lipid-lowering treatment and progression of renal disease.

<table>
<thead>
<tr>
<th>Study (ref)</th>
<th>Intervention</th>
<th>Patients</th>
<th>N, follow-up time</th>
<th>Renal outcome</th>
<th>Additional information</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACCORD (297)</td>
<td>Fenofibrate 160 mg/PBO</td>
<td>T2DM, high vascular risk, all patients received 20-40 mg simvastatin</td>
<td>N=5518, 4.7 years</td>
<td>Incidence of micro 38.2% vs. 41.6% (p=0.01). Incidence of macro 10.5% vs. 12.3% (p=0.04)</td>
<td>Reduced dose of fenofibrate if eGFR &lt;50 ml/min/1.73 m²²</td>
</tr>
<tr>
<td>DAIS (314)</td>
<td>Fenofibrate 200 mg/PBO</td>
<td>T2DM without nephropathy</td>
<td>N=314, 3.3 years</td>
<td>Reduction of progression to micro 3.0% vs. 17.7% (p&lt;0.001)</td>
<td>8% on fenofibrate and 18% on PBO had higher AER at trial end</td>
</tr>
<tr>
<td>FIELD (295)</td>
<td>Fenofibrate 200 mg/PBO</td>
<td>T2DM, majority without overt nephropathy</td>
<td>N=9795, 5 years</td>
<td>2.6% less progression or more regression of albuminuria (p=0.002)</td>
<td>Statistically significant if pooled with regression of albuminuria</td>
</tr>
<tr>
<td>HPS (288)</td>
<td>Simvastatin 40 mg/PBO</td>
<td>T1DM (3%), T2D(26%), arterial disease without DM (71%)</td>
<td>N=20 270, (5963 with DM) 4.6 years</td>
<td>Slower decrease in eGFR 5.9 vs. 6.7 ml/min/1.73 m²² (p=0.0003) during follow-up</td>
<td>Effect on eGFR larger in patients with diabetes</td>
</tr>
<tr>
<td>CARDS (301)</td>
<td>Atorvastatin 10 mg/PBO</td>
<td>T2DM, no prior CVD, 34% impaired eGFR</td>
<td>N=2838, 3.9 years</td>
<td>Net improvement in eGFR 0.18 ml/min/1.73 m²/year</td>
<td>Net improvement 0.38 ml/min/1.73m²/year in those with albuminuria</td>
</tr>
<tr>
<td>TNT (302)</td>
<td>Atorvastatin 10 or 80 mg</td>
<td>T2DM, CAD</td>
<td>N=1431, 4.8 years</td>
<td>Improvement in eGFR at the end of follow-up in both treatment groups 0.5 vs. 2.6 ml/min/1.73 m²² (p=0.001)</td>
<td></td>
</tr>
<tr>
<td>ALERT (307, 308)</td>
<td>Fluvastatin 40 mg/80 mg/PBO</td>
<td>Renal transplant recipients, 13% with DM</td>
<td>N=2102, 6 years</td>
<td>No effect</td>
<td>GFR measured directly in a subset of 439 patients</td>
</tr>
<tr>
<td>SHARP (306)</td>
<td>Simvastatin 20 mg and Ezetimibe 10 mg/PBO</td>
<td>Dialysis or pre-dialysis patients, 20% with DM</td>
<td>N=9270, 4.9 years</td>
<td>No effect</td>
<td>Simvastatin 20 mg alone in 1054 patients for one year</td>
</tr>
</tbody>
</table>

PBO = placebo, T1DM = type 1 diabetes mellitus, T2DM = type 2 diabetes mellitus, micro = microalbuminuria, macro = macroalbuminuria
2.4.2 Lipid-lowering treatment and progression of diabetic retinopathy

Data on the effect of statin treatment on diabetic retinopathy are lacking from large randomized clinical trials, but because statin treatment is such an important part of the prevention of CVD, it would today be considered unethical to have a placebo arm in a trial including patients with increased CVD risk at baseline. Previously, the CARDS showed a trend towards a reduced need of retinal laser treatment with atorvastatin treatment, but no impact on the progression of diabetic retinopathy was seen (290). In contrast, large trials investigating the effect of fibrate therapy on the development and progression of diabetic retinopathy have been performed. In the FIELD Study, fenofibrate treatment significantly reduced the need for the first laser treatment due to either PDR or macular edema (hazard ratio [HR] 0.69). However, the need for laser treatment was not predicted by the baseline plasma lipid concentrations (150). The NNT for prevention of laser treatment in patients with pre-existing retinopathy was fairly low and clinically worthwhile (NNT=17), while the results for primary prevention of diabetic retinopathy were not as convincing (NNT=90). In the FIELD Ophthalmology Substudy, a 2-step progression on the ETDRS severity scale did not differ between the two groups overall, but among patients with pre-existing retinopathy, fewer patients on fenofibrate had a 2-step progression than in the placebo group. In the ACCORD Study, fenofibrate together with simvastatin treatment reduced the rate of progression of diabetic retinopathy, defined as at least a 3-step progression on the ETDRS scale or the development of PDR (HR 0.60) compared with simvastatin treatment alone (167).

2.4.3 Lipid-lowering treatment and risk of development of type 2 diabetes

Cardiovascular risk factors and risk factors for type 2 diabetes are often present in the same patients, therefore, many patients who are prescribed statin treatment already have an increased risk for development of type 2 diabetes before initiation of statin treatment. However, recent data have shown that statin treatment in itself is associated with a modestly increased risk of type 2 diabetes. In a meta-analysis, statin treatment increased the risk of diabetes development by 9% (320). In another meta-analysis, intensive-dose treatment was associated with a 12% increased risk compared with moderate-dose statin treatment, but intensive-dose treatment was also associated with fewer major CVD events (OR 0.84) (31). Simvastatin, atorvastatin and rosuvastatin have all been associated with an increased diabetes risk, but the results regarding pravastatin treatment have been conflicting, and even protective effects have been reported (321). In an analysis from the Jupiter Trial, rosuvastatin treatment was associated with a 39% reduction of the primary CVD endpoint and a 28% increase in diabetes in patients with common risk factors for diabetes, but in patients without risk factors for diabetes no increase in the risk of diabetes development was seen (322). The increased diabetes risk is observed especially in older patient groups. In a Finnish study, less weight loss during statin treatment was observed in
elderly men (median age 73 years), hence, the possible positive protective effect of statin treatment against frailty may paradoxically lead to a higher diabetes risk (323). Further, a Mendelian randomization study showed that genetic variants in the \textit{HMG-CoA} gene associated with lower LDL cholesterol were also associated with an increased risk of type 2 diabetes and higher bodyweight (324). All in all, the risk of diabetes development with statin treatment is fairly low and the cardiovascular benefits outweigh the diabetogenic risk. The guidelines regarding cardiovascular prevention and statin treatment have therefore not been altered. However, patients receiving statin treatment who have risk factors for diabetes should be informed about the risk, receive support for lifestyle changes, and regularly be monitored for hyperglycemia.
3 AIMS OF THE STUDY

The main aims of this thesis were as follows:

I To examine the relationship between lipid variables, eGFR, and AER. A further aim was to assess the effect of glycemic control, obesity, and hypertension on lipid profiles in patients without renal disease.

II To evaluate the impact of baseline lipid values on the progression of renal disease in patients with type 1 diabetes at all stages of albuminuria.

III To investigate the association between lipid variables and diabetic retinopathy in patients with type 1 diabetes. Furthermore, interactions and correlations between diabetic retinopathy, nephropathy, and lipid variables were explored.

IV To assess the ability of lipid variables to predict incident CAD events in patients with type 1 diabetes. Moreover, the effect of renal disease, sex, and glycemic control on the ability of the lipid variables to predict CAD events was explored.
4 SUBJECTS AND STUDY DESIGN

4.1 The FinnDiane Study

These studies are part of the ongoing prospective Finnish Diabetic Nephropathy Study (FinnDiane), a nationwide, multicenter study, initiated in 1997 that recruited its first patients in January 1998. Prior to the FinnDiane Study, two pilot studies, GENREL and NEFREL, that recruited families with diabetic nephropathy existed and the patients from these studies were also included in the FinnDiane population. Follow-up data have been collected since 2004. The aim of FinnDiane is to identify genetic, environmental, and clinical risk factors for micro- and macrovascular complications in patients with type 1 diabetes, with a special emphasis on diabetic nephropathy. The main aims of FinnDiane are to cover ~25% of all adult patients with type 1 diabetes in Finland and to answer the question: why do one-third of patients with type 1 diabetes develop diabetic nephropathy? Adult patients from 77 hospitals and primary healthcare centers in Finland have consecutively been asked to participate in the study, and the response rate has been about 78% (325). The study centers include all 5 university central hospitals, all 16 central hospitals, 26 other hospitals, and 30 primary healthcare units in Finland (listed in the Appendix). The geographic distribution of the FinnDiane patients (Figure 8) closely follows the distribution of the general population in Finland, and the high response rate makes any major significant biases unlikely. At the moment around 5000 patients with type 1 diabetes have been recruited, which represents about 12.5% of all patients with type 1 diabetes in Finland. In each study of this thesis, we started with the inclusion of all patients with centrally measured lipid profiles available from the database at the time of the study and then added the selection criteria for each study, which are explained in more detail below.

Figure 8. Distribution of the FinnDiane patients. Each dot indicates one FinnDiane patient. The distribution is similar to the distribution of the general population in Finland.
4.2 Collection of cross-sectional data

At baseline, patients underwent a thorough clinical investigation at a regular visit to their attending physician. Data on diabetes duration, medication, diabetic microvascular complications, smoking, and cardiovascular disease were registered based on medical records and obtained by the patient’s attending physician using a standardized questionnaire. The baseline data were obtained from patients who participated in the FinnDiane Study between 1994 and 2008.

Study I: At the time of the study, complete lipid profiles were obtained from 3448 patients; the selection criteria are described in Figure 9. The clinical characteristics of the patients grouped by albuminuria status can be seen in Table 3.
Table 3. Clinical characteristics of subjects grouped by albuminuria status.

<table>
<thead>
<tr>
<th></th>
<th>Normal AER</th>
<th>Micro</th>
<th>Macro</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1959</td>
<td>453</td>
<td>515</td>
</tr>
<tr>
<td>Men (%)</td>
<td>48.2</td>
<td>58.5</td>
<td>57.9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35.5 ± 11.9</td>
<td>37.9 ± 11.7</td>
<td>40.9 ± 9.8</td>
</tr>
<tr>
<td>Age at onset (years)</td>
<td>16.2 ± 8.5</td>
<td>11.8 ± 7.9</td>
<td>11.7 ± 7.2</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>19.3 ± 11.6</td>
<td>26.1 ± 10.7</td>
<td>29.2 ± 7.8</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>129 ± 15</td>
<td>135 ± 17</td>
<td>144 ± 19</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78 ± 9</td>
<td>80 ± 10</td>
<td>83 ± 10</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.8 ± 3.3</td>
<td>25.6 ± 3.6</td>
<td>25.8 ± 4.0</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.2 ± 1.4</td>
<td>8.8 ± 1.5</td>
<td>9.0 ± 1.5</td>
</tr>
<tr>
<td>Albumin excretion rate (mg/24h)</td>
<td>8 (5-13)</td>
<td>56 (26-107)</td>
<td>497 (166-1270)</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>83 ± 16</td>
<td>90 ± 19</td>
<td>171 ± 125</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>22.2</td>
<td>29.6</td>
<td>29.1</td>
</tr>
</tbody>
</table>

Data are means ± SD, median (IQR) or %. AER = albumin excretion rate, Micro = microalbuminuria, Macro = macroalbuminuria.

Study III: Retinopathy status was determined in 1465 consecutively recruited patients with complete lipid profiles. To avoid selection bias, these patients represent the first consecutive patients participating in the FinnDiane Study. In addition, an independent data set of 1110 patients without ESRD and a minimum diabetes duration of 10 years was evaluated to replicate the interaction and correlation analyses.

4.3 Collection of follow-up data

Study II: At follow-up, all available medical files, including laboratory data, were reviewed and any changes in renal status were verified. Prospective data were available for 2412 patients. Patients with ESRD (n = 143) were excluded. Altogether, 2304 patients participated in the study and were followed for 5.4 ± 2.0 years. Progression was defined as a change from a lower to a higher level of albuminuria (normal AER to microalbuminuria, or micro- to macroalbuminuria) or development of ESRD in patients with macroalbuminuria at baseline.

Study IV: Complete baseline data, including centrally measured lipid profiles, were available for 3872 patients. Follow-up data were obtained from the Finnish Hospital Discharge Register (HILMO) based on hospital discharge records and the Causes of Death Register through to 31.12.2010 and available for all patients. Patients with acute myocardial infarction, coronary artery bypass graft surgery, or coronary angioplasty at baseline were excluded (N=306). Further, patients with International Classification of
Diseases (ICD)-10 diagnosis codes I20 and I22-25 (ICD-9: 411-414) in the Finnish Hospital Discharge Register, those with reported coronary heart disease, or those taking long-acting nitroglycerin medication at baseline were excluded from the patient group without an event during the follow-up period (N=46). Hence, a total of 3520 patients were included and followed for a median of 10.2 (8.6-12.0) years.

4.4 Ethical aspects

The FinnDiane Study protocol was approved by the Ethics Committee of Helsinki University Central Hospital (decision number: 491/E5/2006) as well as by the local ethics committees of each participating study center and is being conducted in accordance with the Declaration of Helsinki. All patients gave their written informed consent before participation in the study. All research files used for data analyses were coded with ID numbers and personal information is known only to the FinnDiane researchers.

The FinnDiane Study is an observational study; hence, no interventions for patients are carried out. The only potential nuisance to patients is the possible pain caused by venapuncture when blood samples are drawn as well as the extra time spent during study visits and in completing questionnaires.
5 METHODS

5.1 FinnDiane Study protocol

5.1.1 Definition of type 1 diabetes

Type 1 diabetes was defined as onset of diabetes before the age of 35 years and with permanent insulin treatment initiated within one year of diagnosis. Adult patients with type 1 diabetes from 77 hospitals and primary healthcare centers all over Finland were consecutively invited to participate.

5.1.2 Definition of diabetic nephropathy

Nephropathy status was determined based on the measurement of albumin excretion rate (AER) in at least two of the three consecutive 24-h or overnight urine collections.

<table>
<thead>
<tr>
<th>Normal AER: AER&lt;20 μg/min or &lt;30 mg/24 h</th>
<th>Microalbuminuria: 20≤AER&lt;200 μg/min or 30≤AER&lt;300 mg/24 h</th>
<th>Macroalbuminuria: AER≥200 μg/min or AER≥300 mg/24 h</th>
<th>ESRD: Dialysis or kidney transplant</th>
</tr>
</thead>
</table>

5.1.3 Assessment of renal function

Estimated glomerular filtration rate (eGFR) was calculated on the basis of a single serum creatinine measurement using the Cockcroft–Gault formula adjusted for body surface area (71) (Studies I and II), the Modification of Diet in Renal Disease (MDRD-4) equation (72) (Studies II and III), or the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (73) (Study IV).

The Cockcroft-Gault formula is calculated as:

\[
eGFR = \frac{([140\text{-age}] \times \text{weight} [\text{kg}] \times \text{constant})}{\text{creatinine}}
\]

-the constant is 1.23 for men and 1.04 for women

The MDRD-4 equation is calculated as:

\[
eGFR =32788 \times \text{creatinine}^{-1.154} \times \text{age}^{-0.203} \times (0.724 \text{ if female})
\]
The CKD-EPI formula is calculated as:

\[
\begin{align*}
&\text{a) Female S-Creatinine } \leq 61.9 \ \mu\text{mol/l: } e\text{GFR} = 144 \times (\text{creatinine} / 61.9)^{-0.329} \times (0.993)^{\text{age}} \\
&\text{b) Female S-Creatinine } > 61.9 \ \mu\text{mol/l: } e\text{GFR} = 144 \times (\text{creatinine} / 61.9)^{-1.209} \times (0.993)^{\text{age}} \\
&\text{c) Male S-Creatinine } \leq 79.6 \ \mu\text{mol/l: } e\text{GFR} = 141 \times (\text{creatinine} / 79.6)^{-0.411} \times (0.993)^{\text{age}} \\
&\text{d) Male S-Creatinine } > 79.6 \ \mu\text{mol/l: } e\text{GFR} = 141 \times (\text{creatinine} / 79.6)^{-1.209} \times (0.993)^{\text{age}}
\end{align*}
\]

In Study I, patients were divided into three groups on the basis of the eGFR calculated with the Cockcroft–Gault formula:

\[
\begin{align*}
&\text{Normal renal function: } >90 \ \text{ml/min/1.73 m}^2 \\
&\text{Mildly impaired renal function: } 60-90 \ \text{ml/min/1.73 m}^2 \\
&\text{Impaired renal function: } <60 \ \text{ml/min/1.73 m}^2
\end{align*}
\]

5.1.4 Definition of diabetic retinopathy

Fundus photographs taken by the participating study centers were scanned and stored in a digital archive; these were available for 1128 (77%) of 1465 patients. Ophthalmic records with information about fundus examinations were also obtained. The clinical fundus examination is important because it has good specificity (326) and the combination of fundus photographs and a clinical examination provide both good sensitivity and specificity for detection of severe diabetic retinopathy. The data were analyzed by a specialist in ophthalmology (Kustaa Hietala) who was unaware of the demographic data and the presence of other complications. The ETDRS scale was used to grade the severity of diabetic retinopathy, with 10 defined as no diabetic retinopathy, 20-35 as mild non-proliferative diabetic retinopathy (NPDR), 43-53 as moderate to severe NPDR, and 61 and over as proliferative diabetic retinopathy (PDR) (327). The eye with the more severe diabetic retinopathy was used to determine the retinopathy stage for a patient. For patients without available fundus photographs the verbal descriptions of clinical fundus examinations by ophthalmologists were transformed to approximate numerical values on the ETDRS scale. In an independent cohort in Study III, severe diabetic retinopathy was defined as history of laser photocoagulation. The underlying cause for laser treatment was PDR in the majority (>80%) of patients, and the rest of the patients received laser photocoagulation mainly due to macular edema or severe non-proliferative retinopathy (161).

ETDRS severity scale:

\[
\begin{align*}
&\text{No retinopathy: Level 10} \\
&\text{Mild NPDR: Level 20-35} \\
&\text{Moderate to severe NPDR: Level 43-53} \\
&\text{PDR: Level } \geq 61
\end{align*}
\]
5.1.5 Definition of cardiovascular disease (CVD)

A CVD event was defined as a history of myocardial infarction, stroke (cerebral infarction or intracerebral hemorrhage), or amputation. In Study I, coronary heart disease (CHD) was defined as myocardial infarction, coronary revascularization, or regular use of long-acting nitroglycerin.

5.1.6 Definition of coronary artery disease (CAD)

In Study IV, an incident CAD event was defined as myocardial infarction given as ICD-10 code I21 (ICD-9: 410), coronary artery bypass graft surgery, or coronary angioplasty. Author Nina Tolonen from the FinnDiane Study group verified the Finnish Hospital Discharge Register (HILMO) data by reviewing the hospital records of 28% of the patients. In this sample, no typing errors were found, and only four borderline cases of acute myocardial infarction were identified. Otherwise, all cases were in accordance with the universal definition of myocardial infarction (328) or had undergone either coronary artery bypass graft surgery or coronary angioplasty. Fatal CAD events were identified from a search of the Finnish Causes of Death Register and established when the immediate or underlying cause of death was from CAD, i.e. given as ICD-10: I20-25 (ICD-9: 410-414). Death certificates were also obtained to verify the register data.

5.1.7 Anthropometric measurements

Weight and height were recorded with 0.1 kg and 1 cm accuracy, respectively. Waist circumference was measured halfway between the lowest rib and the iliac crest. Hip circumference was measured at the major trochanters of the femurs. Waist-to-hip ratio (WHR) was calculated by dividing the waist circumference by the hip circumference. Body mass index (BMI) was calculated as weight/height² (kg/m²).

In Study I, patients were divided into three groups based on their BMI:

- **Normal BMI:** < 25 kg/m²
- **Overweight:** 25–30 kg/m²
- **Obesity:** > 30 kg/m²

5.1.8 Assessment of blood pressure

Blood pressure was measured twice from the brachial artery at 2-min intervals in the sitting position after a 10-min rest. A manual sphygmomanometer or an automated blood pressure measurement device was used. The mean value of at least two measurements was used in the analyses.
In Study I, hypertension was defined as the use of antihypertensive medication or systolic/diastolic blood pressure higher than 130/80 mmHg (116).

5.1.9 Definition of smoking

Current smoking was defined as smoking at least one cigarette per day at the time of data collection. History of smoking was defined as smoking at least one cigarette per day for a minimum of 3 months but ceasing to smoke before data collection.

5.2 Laboratory measurements and assays

5.2.1 Lipids and lipoproteins

All serum lipid and lipoprotein concentrations were measured from blood samples in Professor Marja-Riitta Taskinen’s research laboratory at Helsinki University Central Hospital, Division of Cardiology, Helsinki, Finland. Total cholesterol and triglycerides were determined enzymatically using a Cobas Mira analyzer (Hoffman-La Roche, Basel, Switzerland) with commercially available kits (Hoffman-La Roche until November 2001 and ABX Diagnostics, Montpellier, France until January 2006). Thereafter, an enzymatic determination by Konelab 60i analyzer (Thermo Fisher Scientific, Waltham, MA, USA) and a kit from the same manufacturer were used. Total HDL and HDL$_3$ cholesterol were determined enzymatically using a HTS 7000 Plus Bio Assay Reader (Orion Diagnostica, Espoo, Finland) until January 2006. Thereafter, an immunoprecipitation method with a Konelab 60i analyzer and a kit from the same manufacturer (Thermo Fisher Scientific) were used. Serum ApoB concentrations were determined using a Cobas Mira analyzer by immunoprecipitation with a commercial kit (Orion Diagnostica, Espoo, Finland) until January 2006. Thereafter, an immunoprecipitation method with a Konelab 60i analyzer and a kit from the same manufacturer (Thermo Fisher Scientific) were used. Serum ApoA-I concentrations were determined with a Cobas Mira analyzer by immunoprecipitation with commercial kits (Boehringer-Mannheim until January 2002 and Wako Chemicals GmbH, Neuss, Germany until January 2006), and thereafter, with a Konelab 60i analyzer by immunoprecipitation (Thermo Fisher Scientific). Serum ApoA-II concentrations were determined with a Cobas Mira analyzer by immunoprecipitation with a commercial kit (Boehringer-Mannheim until August 2001), and thereafter, a polyclonal antibody produced in sheep against human ApoA-II was used.
In Study I, cut-off values based on the recommendation of the American Diabetes Association (116) were as follows: LDL cholesterol ≤2.6 mmol/l, triglycerides ≤1.7 mmol/l, and HDL cholesterol ≥1.0 mmol/l for men and ≥1.3 mmol/l for women.

5.2.2 HbA₁c

Glycosylated hemoglobin A₁c (HbA₁c) was determined locally at each center by standardized assays. In Study I, patients were divided into three groups with regard to glycemic control:

<table>
<thead>
<tr>
<th>Good glycemic control:</th>
<th>Intermediate glycemic control:</th>
<th>Poor glycemic control:</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA₁c &lt; 7.5%</td>
<td>HbA₁c 7.5-9%</td>
<td>HbA₁c &gt; 9%</td>
</tr>
</tbody>
</table>

5.2.3 Assessment of insulin sensitivity

To estimate insulin sensitivity, estimated glucose disposal rate (eGDR) was calculated with an equation developed by Williams et al. (124) based on clinical risk factors and validated with euglycemic-hyperinsulinemic clamp measurements in a subset of the Pittsburgh EDC Study population. An equation modified for use with HbA₁c instead of HbA₁ was used in this study.

\[
eGDR = 24.4 - 12.97 \times \text{WHR} - 3.39 \times \text{hypertension} - 0.60 \times \text{HbA₁c}
\]

Hypertension is defined as antihypertensive treatment and/or blood pressure ≥140/90 mmHg (yes = 1, no = 0).

5.2.4 Creatinine

Serum creatinine was measured with a kinetic Jaffé reaction using a Hitachi 911 E analyser (Boehringer Mannheim, Mannheim, Germany) until January 2002. Thereafter, a photometric, enzymatic (isotope dilution mass spectrometry = IDMS) method using a Hitachi 917 or Modular analyser (Boehringer Mannheim/Roche Diagnostics, Basel, Switzerland) was applied. The correlation coefficient between the two methods is 0.988.

To enable use of the data derived from both methods, the following conversion formula was applied:

\[
\text{S-Creatinine (IDMS)} = (0.953 \times \text{S-Creatinine Jaffé}) - 7.261
\]
5.2.5 Urinary albumin excretion rate (AER)

In addition to the urine collections used for the classification of renal status, AER was also measured centrally from 24-h urine collections. It was measured by radioimmunoassay using a LKB Wallac RiaGamma counter (Pharmacia, Uppsala, Sweden) until November 2002. Thereafter, an immunoturbidimetric method was used with a Hitachi 911 analyzer (Roche Diagnostics, Hoffman-La Roche, Basel, Switzerland). This measurement was included in the multivariate analyses.

5.3 Lipid-lowering medication

Lipid-lowering medication was defined as the use of statins, fibrates, and/or ezetimibe; however, only a few patients were on fibrate or ezetimibe therapy. The active substance and doses of patients’ lipid-lowering medication were registered. In Study I, patients with lipid-lowering medication were excluded from the analyses. In the other studies, parts of the analyses were performed correcting for or excluding patients with lipid-lowering medication.

5.4 Statistical analyses

Data for normally distributed and continuous variables are presented as mean ± standard deviation (SD) and data for non-normally distributed variables as median with interquartile range (IQR). Differences between groups were analyzed with Student’s t-test or ANOVA for normally distributed variables between two or three groups, respectively. Differences between non-normally distributed variables for two groups were analyzed with the Mann-Whitney U-test and for three groups with the Kruskal-Wallis test. Categorical variables were analyzed using Pearson’s \( \chi^2 \) test. Values of \( p \) for lipid variables for comparison between groups were adjusted for age, sex, and BMI. Pearson’s correlation coefficients were used to calculate correlations between normally distributed values, and Spearman’s rank correlation coefficients were used for non-normally distributed values. Non-normally distributed values were logarithmically transformed before inclusion in the multivariate models.

In Study I, multiple linear regression analyses were performed with either eGFR or AER as the dependent variable.

5.4.1 Study II

Cox proportional hazards model was used to investigate the relationship between possible predictors of progression of diabetic nephropathy. Results are presented as hazard ratios (HRs) with 95% confidence intervals (CIs). A standard model, including conventional risk
factors, i.e. diabetes duration, HbA$_{1c}$, SBP, sex, BMI, and current smoking, was used for the analyses. Receiver-operating characteristic (ROC) curves were performed to identify possible thresholds of triglycerides for the prediction of renal disease. The shortest distance on the ROC curve corresponding to the maximum sum of sensitivity and specificity was used in the determination of cut-off points.

5.4.2 Study III

PDR or mild NPDR was the dependent variable in multiple logistic regression analyses. To determine whether the lipid variables have a different effect on AER depending on retinopathy status, interaction terms between retinopathy status (no retinopathy, mild NPDR, moderate to severe NPDR, PDR) and lipid variables were explored with linear regression models where the natural logarithm of (ln)AER was the dependent variable. The relationship between lipid variables and retinopathy status was further analyzed with least square estimates for ln AER, which were calculated after stratification of the data by quartiles of triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, and retinopathy status groups. Back-transformation resulted in geometric means adjusted for diabetes duration, SBP, and HbA$_{1c}$ (Figure 1 in Study III). Patients with ESRD and a diabetes duration of less than 10 years were excluded from correlation and interaction analyses and from Figure 1. In the additional correlation and interaction analyses in an independent data set, retinal laser treatment (yes, no) was used to stratify the cohort. Patients with ESRD and diabetes duration of less than 10 years were also excluded from these analyses.

5.4.3 Study IV

To analyze the associations between risk factors and incident CAD events, univariate and multivariate Cox regression models were used. Variables included in the multivariate models were all univariately associated with CAD and every variable reduced the Akaike information criteria (AIC), except for sex, but since it is a well-established CAD risk factor, it was included in the models. The multivariate models included diabetes duration, HbA$_{1c}$, SBP, sex, WHR, eGFR, retinal laser treatment, AER, history of smoking, and one of the lipid variables. Because of collinearity, only one of the lipid variables was entered into the models at a time. Results are presented as HRs per SD increase, with 95% CIs. The standardized score for WHR was calculated separately for men and women. Fine and Gray regression analyses were also performed to take into account the competing event of non-CAD death (330). After the Fine and Gray competing risks analyses were performed, figures of the cumulative incidence for CAD in normoalbuminuric or macroalbuminuric patients divided by the median of lipid variables were drawn. To compare the ability of the lipid variables to predict an incident CAD event, we calculated the area under the ROC curves (AUC). Further, likelihood ratio (LR) $\chi^2$ statistics from the Cox models were calculated, a higher value indicating a better global fit. Net reclassification improvement
(NRI) is the percentage reclassified after the inclusion of the variable of interest in the above-mentioned multivariate model, distinguishing between movements in the correct direction, i.e. the proportion of subjects being reclassified to a higher risk category amongst CAD cases or a lower risk category amongst controls (331). The 5%, 10%, and 20% cut-off points have been proposed as relevant in clinical-decision making for CAD prevention (332, 333) and were therefore chosen as the NRI cut-off points.

In the multivariate models in Studies I and II, a p-value of < 0.05 was considered significant. Otherwise, a more stringent level of significance (p<0.01) was chosen in order to correct for multiple testing. Statistical analyses were performed using SPSS 12.0.1, 15.0, PASW Statistics 18 for Windows (SPSS Inc., Chicago, IL, USA), Statistical Analysis System version 9.2 (SAS Institute, Cary, NC, USA), STATA Data Analysis and Statistical Software (StataCorp LP, College Station, TX, USA), and MedCalc (MedCalc Software bvba, Ostend, Belgium).
6 RESULTS

6.1 Associations between lipid profiles, AER, and eGFR (Study I)

Patients with impaired renal function had higher total cholesterol, LDL cholesterol, triglycerides, and ApoB and lower HDL cholesterol than patients with normal or only mildly impaired renal function (Table 4). The lipid profiles of patients with mildly impaired renal function were similar to those with normal renal function.

When patients were divided by their albuminuria status, lipid abnormalities could be seen already at the microalbuminuric stage for total cholesterol, triglycerides, and ApoB (p<0.001 for all). In macroalbuminuric patients, the lipid disturbances were further altered with higher total cholesterol, LDL cholesterol, triglycerides, and ApoB as well as with lower HDL, HDL₂, and HDL₃ cholesterol than in both normo- and microalbuminuric patients (p<0.001 for all).

Table 4. Lipid profile stratified by estimated glomerular filtration rate (eGFR).

<table>
<thead>
<tr>
<th></th>
<th>eGFR &gt;90</th>
<th>eGFR 60-90</th>
<th>eGFR &lt;60</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1505</td>
<td>857</td>
<td>228</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.80 ± 0.95</td>
<td>5.04 ± 0.84</td>
<td>5.37 ± 1.10‡*</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mmol/l)</td>
<td>3.51 ± 0.96</td>
<td>3.64 ± 0.89</td>
<td>4.12 ± 1.04‡*</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>2.98 ± 0.86</td>
<td>3.14 ± 0.80</td>
<td>3.40 ± 0.92‡*</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.00 (0.76-1.41)</td>
<td>0.95 (0.74-1.28)</td>
<td>1.38 (1.04-2.10)‡*</td>
</tr>
<tr>
<td>Apolipoprotein B (g/l)</td>
<td>0.87 ± 0.22</td>
<td>0.88 ± 0.21†</td>
<td>0.99 ± 0.25‡*</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.27 ± 0.34</td>
<td>1.40 ± 0.38</td>
<td>1.19 ± 0.39‡*</td>
</tr>
<tr>
<td>HDL₂ cholesterol (mmol/l)</td>
<td>0.50 ± 0.23</td>
<td>0.61 ± 0.27</td>
<td>0.49 ± 0.26‡*</td>
</tr>
<tr>
<td>HDL₃ cholesterol (mmol/l)</td>
<td>0.77 ± 0.18</td>
<td>0.79 ± 0.21</td>
<td>0.71 ± 0.20‡*</td>
</tr>
<tr>
<td>Apolipoprotein A-I (g/l)</td>
<td>1.36 ± 0.21</td>
<td>1.44 ± 0.22†</td>
<td>1.37 ± 0.23‡*</td>
</tr>
</tbody>
</table>

Data are means ± SD or median (IQR). †p<0.01, ‡p<0.001 vs. eGFR >90; *p<0.001 vs. eGFR 60–90. Data are adjusted for age, body mass index, and sex.

Factors associated with eGFR and AER in multiple linear regression models

To study factors associated with eGFR, multiple linear regression analysis was performed. Age, BMI, ApoB, and SBP were independently associated with eGFR (R²=0.28) (Table 5). When ApoB was replaced with triglycerides and LDL cholesterol, triglycerides were also independently associated with eGFR (R²=0.28). When AER was added to the model with ApoB, systolic blood pressure and ApoB were no longer independently associated, but HDL cholesterol emerged as a new independently associated factor together with age, BMI, and AER (R²=0.36).
Table 5. Factors associated with eGFR in a multiple linear regression analysis.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>SE</th>
<th>β</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-1.17</td>
<td>0.05</td>
<td>-0.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>-0.13</td>
<td>0.03</td>
<td>-0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>2.18</td>
<td>0.15</td>
<td>0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apolipoprotein B (g/l)</td>
<td>-10.39</td>
<td>2.34</td>
<td>-0.08</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

R²=0.28. Patients with lipid-lowering treatment were excluded. The model also included HDL cholesterol. eGFR= estimated glomerular filtration rate, B = unstandardized regression coefficient, SE = standard error of B, β = standardized regression coefficient.

SBP, HbA₁c, diabetes duration, ApoB, and HDL cholesterol were independently associated with AER (R²=0.23) (Table 6). When ApoB was replaced with triglycerides and LDL cholesterol, they were also independently associated with AER (R²=0.23). When eGFR was added to the model with ApoB, it was also an independent factor for AER together with all of the other variables in the model (R²=0.27).

Table 6. Factors associated with ln AER in multiple linear regression analysis.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>SE</th>
<th>β</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes duration (years)</td>
<td>0.02</td>
<td>0.003</td>
<td>0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0.02</td>
<td>0.002</td>
<td>0.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>0.23</td>
<td>0.02</td>
<td>0.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>-0.39</td>
<td>0.09</td>
<td>-0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apolipoprotein B (g/l)</td>
<td>1.29</td>
<td>0.16</td>
<td>0.17</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

R²=0.23. Patients with lipid-lowering treatment were excluded. Ln AER= natural logarithm of albumin excretion rate, B = unstandardized regression coefficient, SE = standard error of B, β = standardized regression coefficient.

The relationship between eGFR and AER can be seen in Figures 10A and 10B.
Figure 10. A) Frequency of normal (eGFR >90), mildly impaired (eGFR 60-90), or impaired (eGFR <60) renal function when patients are stratified by albuminuria status. B) Frequency of normal albumin excretion rate (normo), microalbuminuria (micro), or macroalbuminuria (macro) when patients are stratified by renal function. eGFR= estimated glomerular filtration rate.
Prevalence of patients achieving the targets for lipid variables
During the time of the data collection, 1994-2004, two targets for LDL cholesterol were in use. In patients without manifest renal disease who had good glycemic control, normal blood pressure, or normal body weight, only 51% achieved the criteria for an LDL cholesterol target of $\leq 3.0$ mmol/l and merely 41% the more stringent criteria of $\leq 2.6$ mmol/l. In patients with renal disease, the treatment targets were achieved by even fewer patients (Figure 11).

![Figure 11. Prevalence of patients stratified by their albuminuria status reaching recommended targets of LDL cholesterol ($\leq 3.0$ mmol/l) HDL cholesterol ($\geq 1.0$ mmol/l for men, $\geq 1.3$ mmol/l for women), and triglycerides ($\leq 1.7$ mmol/l). Normo = normal albumin excretion rate, micro = microalbuminuria, macro = macroalbuminuria, -C= cholesterol, TG= triglycerides.]

6.2 Prediction of progression of renal disease by lipid profiles (Study II)
During a follow-up of $5.4 \pm 2.0$ years, 100 patients developed microalbuminuria, 50 progressed from micro- to macroalbuminuria, and 92 progressed from macroalbuminuria to ESRD. Hence, 242 (10.5%) of 2304 patients progressed to a higher level of albuminuria or developed ESRD.

Patients who developed microalbuminuria or progressed from micro- to macroalbuminuria had higher total cholesterol, non-HDL cholesterol, triglycerides, ApoB, and triglyceride/HDL cholesterol ratio ($p<0.001$ for all) at baseline than patients who did not progress.

Progressors from macroalbuminuria to ESRD had higher total cholesterol, LDL cholesterol, non-HDL cholesterol, triglycerides, ApoB, ApoB/ApoA-I, and triglyceride/HDL cholesterol ratio ($p<0.001$ for all), as well as lower HDL, HDL$_2$, HDL$_3$. 64
HDL$_2$/HDL$_3$ cholesterol ratio and ApoA-II concentrations (p<0.01 for all) than patients who did not progress.

**Lipid variables as independent predictors for progression of renal disease**

In a Cox regression analysis, HbA$_{1c}$, male sex, and triglycerides were independent predictors of development of microalbuminuria and progression to macroalbuminuria. When triglycerides were replaced with the other lipid variables one at a time, ApoB was also an independent predictor of progression to both micro- and macroalbuminuria. When AER was added to the original models, triglycerides were no longer an independent predictor of progression to micro- or macroalbuminuria. However, when the two groups were pooled; HbA$_{1c}$, male sex, triglycerides, and AER were all independent predictors of progression of renal disease.

High SBP, low BMI, and high triglycerides were predictive of progression from macroalbuminuria to ESRD. Total cholesterol, non-HDL cholesterol and triglyceride/HDL cholesterol ratio were also strong predictors of progression to ESRD (p<0.001). However, when baseline eGFR was included in the models, only total cholesterol predicted progression to ESRD together with HbA$_{1c}$ and eGFR.

When ROC curves were created no thresholds for triglycerides and progression of renal disease were identified. To determine at which triglyceride concentration the risk for renal disease progression increases, we also prepared predictive probability plots for progression from normo- to microalbuminuria and from macroalbuminuria to ESRD (Figure 12). The risk of progression increased linearly until a triglyceride concentration of 4 mmol/l was reached and no clinically relevant thresholds for triglycerides were seen.

![Predictive probability plots for triglyceride concentrations and progression from normal AER to microalbuminuria.](Figure 12A)
6.3 Associations between lipid variables, diabetic retinopathy, and nephropathy (Study III)

Of 1465 patients, 381 had no signs of retinopathy, 405 had mild NPDR, 186 had moderate to severe NPDR, and 493 had PDR. Total cholesterol, non-HDL cholesterol, LDL cholesterol, triglycerides, and ApoB were higher in patients with PDR and NPDR than in patients without retinopathy (p<0.001 for all). HDL, HDL\textsubscript{2}, and HDL\textsubscript{3} cholesterol were also lower in patients with PDR than in patients without retinopathy or with NPDR in both men and women (p<0.001 for all).

Diabetes duration and ln AER were positively associated, whereas eGFR and HDL cholesterol (odds ratio [OR] 0.45 [95% CI 0.27-0.74] p=0.002, R\textsuperscript{2}=0.48) were negatively associated with PDR in logistic regression analysis. When HDL cholesterol was replaced with other lipid variables one at a time, HDL\textsubscript{2} cholesterol (OR 0.29 [95% CI 0.14-0.60] p=0.001, R\textsuperscript{2}=0.48) was also inversely associated with PDR. Diabetes duration, HbA\textsubscript{1c}, ln AER, and ln triglycerides (OR 1.86 [95% CI 1.18-2.93] p=0.008, R\textsuperscript{2}=0.44) were positively associated and eGFR was negatively associated with mild NPDR. Patients with PDR or moderate to severe NPDR were excluded from these analyses. When triglycerides were replaced with the other lipid variables, ln triglyceride/HDL cholesterol ratio (OR 1.62 [95% CI 1.14-2.31] p=0.008, R\textsuperscript{2}=0.44) was also positively associated with mild NPDR.
When patients without any signs of renal disease (i.e. normal AER or eGFR >60ml/min/1.73 m²) were analyzed separately, total cholesterol/HDL cholesterol ratio differed significantly between the retinopathy status groups (p=0.007). Trends towards differences in total cholesterol, non-HDL, HDL and HDL₃ cholesterol and triglyceride/HDL cholesterol ratio could also be seen (p<0.05).

Significant interactions between retinopathy status and triglycerides, non-HDL cholesterol, ApoB, total cholesterol/HDL cholesterol ratio, and triglyceride/HDL cholesterol ratio (p<0.001 for all) as well as total cholesterol (p=0.006) were found when interaction terms between retinopathy status (no retinopathy, mild NPDR, moderate to severe NPDR, PDR) and lipid variables were calculated in linear regression models with ln AER as the dependent variable. Further, no significant correlations between the lipid variables and AER were seen in patients without diabetic retinopathy, whereas the correlations between AER and most of the lipid variables were strong in patients with moderate to severe NPDR or PDR (Table 7).

**Table 7.** Spearman correlations between AER and lipid variables according to retinopathy status.

<table>
<thead>
<tr>
<th></th>
<th>No retinopathy</th>
<th>Mild NPDR</th>
<th>Moderate to severe NPDR</th>
<th>PDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (men/women)</td>
<td>141 (64/77)</td>
<td>320 (137/183)</td>
<td>144 (97/47)</td>
<td>318 (160/158)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>NS</td>
<td>NS</td>
<td>0.20 (p=0.02)</td>
<td>0.21 (p&lt;0.001)</td>
</tr>
<tr>
<td>Non-HDL cholesterol</td>
<td>NS</td>
<td>NS</td>
<td>0.27 (p=0.001)</td>
<td>0.24 (p&lt;0.001)</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>NS</td>
<td>NS</td>
<td>0.17 (p=0.04)</td>
<td>0.14 (p=0.02)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>NS</td>
<td>0.18 (p=0.001)</td>
<td>0.35 (p&lt;0.001)</td>
<td>0.36 (p&lt;0.001)</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>NS</td>
<td>0.16 (p=0.004)</td>
<td>0.36 (p&lt;0.001)</td>
<td>0.25 (p&lt;0.001)</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>NS</td>
<td>NS</td>
<td>-0.21 (p=0.01)</td>
<td>-0.17 (p=0.002)</td>
</tr>
<tr>
<td>Apolipoprotein A-I</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Total chol/HDL chol</td>
<td>NS</td>
<td>NS</td>
<td>0.29 (p&lt;0.001)</td>
<td>0.26 (p&lt;0.001)</td>
</tr>
<tr>
<td>Triglyceride/HDL chol</td>
<td>NS</td>
<td>0.14 (p=0.01)</td>
<td>0.33 (p&lt;0.001)</td>
<td>0.34 (p&lt;0.001)</td>
</tr>
<tr>
<td>Apolipoprotein B/A-I</td>
<td>NS</td>
<td>NS</td>
<td>0.29 (p&lt;0.001)</td>
<td>0.18 (p=0.001)</td>
</tr>
</tbody>
</table>

Patients with duration of diabetes less than 10 years and patients with end-stage renal disease were excluded. AER = albumin excretion rate, NPDR = non-proliferative diabetic retinopathy, PDR = proliferative diabetic retinopathy, Chol = cholesterol, NS = non-significant.
6.4 Ability of lipid variables to predict incident CAD events (Study IV)

Of 3520 patients, 310 (9%) suffered an incident CAD event during a median of 10.2 (8.6-12.0) years of follow-up. In general, patients who had an incident CAD event were older, had a longer duration of diabetes, higher SBP, WHR, and AER as well as lower eGDR and eGFR. Of the lipid variables, total cholesterol, non-HDL cholesterol, LDL cholesterol, triglycerides, and ApoB were higher and HDL cholesterol was lower in patients with a CAD event than in those without (Table 8).

Table 8. Lipid profile stratified by an incident coronary artery disease (CAD) event.

<table>
<thead>
<tr>
<th></th>
<th>No CAD event</th>
<th>CAD event</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>3,037</td>
<td>310</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.86 ± 0.92</td>
<td>5.42 ± 1.20‡</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mmol/l)</td>
<td>3.51 ± 0.96</td>
<td>4.20 ± 1.23‡</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>2.97 ± 0.83</td>
<td>3.45 ± 1.00‡</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.98 (0.74-1.39)</td>
<td>1.30 (0.97-1.89)‡</td>
</tr>
<tr>
<td>Apolipoprotein B (g/l)</td>
<td>0.86 ± 0.22</td>
<td>1.00 ± 0.25‡</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l): Men</td>
<td>1.24 ± 0.34</td>
<td>1.14 ± 0.34‡</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.46 ± 0.39</td>
<td>1.33 ± 0.41‡</td>
</tr>
<tr>
<td>HDL&lt;sub&gt;2&lt;/sub&gt; cholesterol (mmol/l): Men</td>
<td>0.47 ± 0.24</td>
<td>0.44 ± 0.23‡</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.64 ± 0.28</td>
<td>0.57 ± 0.28‡</td>
</tr>
<tr>
<td>HDL&lt;sub&gt;3&lt;/sub&gt; cholesterol (mmol/l): Men</td>
<td>0.78 ± 0.19</td>
<td>0.70 ± 0.18‡</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.83 ± 0.22</td>
<td>0.76 ± 0.22‡</td>
</tr>
<tr>
<td>Apolipoprotein A-I (g/l): Men</td>
<td>1.32 ± 0.20</td>
<td>1.32 ± 0.20*</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.46 ± 0.23</td>
<td>1.41 ± 0.23†</td>
</tr>
<tr>
<td>Apolipoprotein B/A-I</td>
<td>0.63 ± 0.20</td>
<td>0.75 ± 0.23‡</td>
</tr>
<tr>
<td>Total cholesterol/HDL cholesterol</td>
<td>3.58 (2.91-4.46)</td>
<td>4.30 (3.55-5.60)‡</td>
</tr>
<tr>
<td>Triglyceride/HDL cholesterol</td>
<td>0.76 (0.51-1.17)</td>
<td>1.06 (0.73-1.90)‡</td>
</tr>
</tbody>
</table>

Data are means ± SD or median (IQR). †p<0.01, ‡p<0.001. P-values are adjusted for age, body mass index, and sex (if not already stratified by sex). * Apolipoprotein A-I concentrations in men were 1.27 and 1.33 g/l in no CAD and CAD event groups, respectively, when corrected for age and body mass index, and this difference was significant.

To take into account the competing event of non-CAD death, we performed Fine and Gray regression analysis (Table 9) in addition to the Cox regression analysis. Duration of diabetes, eGFR, ApoB, SBP, and laser treatment were all independent predictors of CAD in both analyses. In the entire cohort, ApoB, triglycerides, non-HDL cholesterol, ApoB/ApoA-I ratio, and triglyceride/HDL cholesterol ratio were the strongest lipid predictors of an incident CAD event.

68
Table 9. Competing risk regression analysis with risk factors for an incident CAD event.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>SubHR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR (28 ml/min/1.73 m²)</td>
<td>0.69 (0.58 - 0.84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes duration (11.8 years)</td>
<td>2.39 (2.01 - 2.84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apolipoprotein B (0.23 g/l)</td>
<td>1.40 (1.19 - 1.64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Laser treatment (yes, no)</td>
<td>1.61 (1.15 - 2.25)</td>
<td>0.005</td>
</tr>
<tr>
<td>Systolic blood pressure (18 mmHg)</td>
<td>1.22 (1.06 - 1.40)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Results are presented as subdistribution hazard ratios (sub)HRs per SD increase with 95% CI. The model also included HbA1c, sex, waist-to-hip ratio, natural logarithm of albumin excretion rate, and history of smoking. Coronary artery disease (CAD) events = 198, controls = 2219 and non-CAD deaths = 104.

The percentage of patients with a history of smoking was high, and this could be a confounding factor. History of smoking at baseline was univariately associated with CAD, whereas current smoking was not, and therefore, we chose to include history of smoking in the multivariate models. In the multivariate model, history of smoking was not independently associated with CAD events in the entire cohort, unlike several of the lipid variables. We also performed additional NRI analyses to look at the predictive value of history of smoking versus ApoB. The NRI was 0.6% (p=0.62) when we added history of smoking to the multivariate model (including duration of diabetes, eGFR, SBP, retinal laser treatment, sex, HbA1c, WHR, AER, and ApoB), whereas it was 7.7% (p=0.01) when ApoB was added to the model.

Different lipid variables predicted an incident CAD event when patients were divided by sex, renal status, and HbA1c. In men, ApoB was the only lipid variable that was an independent predictor of CAD, whereas in women triglycerides, ApoB/ApoA-I ratio, and triglyceride/HDL cholesterol ratio were the strongest predictors. In macroalbuminuric patients, ApoB and non-HDL, LDL, and total cholesterol were the strongest predictors, whereas ApoB/ApoA-I ratio, triglyceride/HDL cholesterol ratio, triglycerides, and ApoA-I preformed the best in patients with normal AER. ApoB/ApoA-I, triglyceride/HDL cholesterol ratio, and triglycerides were also the best predictors in patients with an HbA1c below the median of the cohort (8.3%), whereas in patients with an HbA1c above the median, the same lipid variables as in the macroalbuminuric patients (ApoB, non-HDL cholesterol, LDL cholesterol, and total cholesterol) were the strongest predictors.

To examine clustering of risk factors, we divided the patients into five groups according to the number of risk factors present. In Cox regression analysis with CAD events as the dependent and clustering as the independent variable, the HR was not significantly different from that of the reference group (i.e. those patients without any or with only one of the five risk factors) for patients with no more than two of any of the five risk factors.
However, in patients with three or more risk factors, the rise in hazard ratio was clear (Figure 13).

Figure 13. Cox regression analysis for clusters of risk factors for CAD events. Patients were stratified into groups according to the presence of any of the five risk factors listed below at baseline: 1) Hypertension, defined as either a systolic blood pressure >140 mmHg or a diastolic blood pressure >80 mmHg (117), 2) presence of renal disease, defined as presence of microalbuminuria, macroalbuminuria, or end-stage renal disease, or an estimated glomerular filtration rate <60 ml/min/1.73 m$^2$, 3) exceeding the recommended HbA$_{1c}$ >7.0% (117), 4) current smoking, or 5) dyslipidemia, defined as total cholesterol >5.0 mmol/l, LDL cholesterol >2.6 mmol/l, triglycerides >1.7 mmol/l, HDL cholesterol <1.0 mmol/l for men, HDL cholesterol <1.3 mmol/l for women (117, 334), or apolipoprotein B > 0.90 g/l (335).
7 DISCUSSION

7.1 Association between renal disease and lipid profiles

7.1.1 Lipid profiles and eGFR

Patients with type 1 diabetes without complications and good glycemic control often have similar or even more favorable lipid profiles than the background population (336, 337). However, we found that patients with type 1 diabetes with an impaired renal function (eGFR<60 ml/min/1.73 m\(^2\)) had higher triglycerides, total cholesterol, LDL cholesterol, and ApoB, and lower HDL cholesterol than patients with eGFR above 60 ml/min/1.73 m\(^2\). Triglycerides were an independent predictor of eGFR in a multiple linear regression model, whereas LDL cholesterol and HDL cholesterol were not. When triglycerides and LDL cholesterol were replaced with ApoB, it was also an independent predictor of eGFR. Previous studies are scarce, but after the publication of our study, associations between lower HDL cholesterol and impaired renal function were observed in patients with type 1 or type 2 diabetes (338). Higher triglycerides were also seen in patients with type 2 diabetes. In patients with type 2 diabetes, but without macroalbuminuria, higher ApoB/LDL cholesterol ratio and ApoC-III concentrations have been associated with impaired renal function regardless of the presence of microalbuminuria (339). In a Korean study of the general population with 93 228 participants, triglycerides and triglyceride/HDL cholesterol ratio were the strongest lipid variables associated with impaired renal function in men, whereas in women LDL cholesterol and non-HDL cholesterol showed the highest ORs (340).

7.1.2 Lipid profiles and AER

The association between lipid variables and AER in patients with type 1 diabetes has been examined to a larger extent. In line with our study, triglycerides and total cholesterol were found to be associated with macroalbuminuria in the DCCT/EDIC, EURODIAB and Estudio Diamante cohorts (144, 341, 342). LDL cholesterol was not reported in the Estudio Diamante Study, but in the other two studies it was also significantly associated with macroalbuminuria. In our study, HDL cholesterol was significantly lower in patients with macroalbuminuria, whereas no significant differences were seen between patients with normal AER or microalbuminuria. In the EURODIAB Study, an association between macroalbuminuria and HDL cholesterol was only seen in women and in the DCCT/EDIC Study, HDL cholesterol was not associated with AER in a multivariate model. We found that triglycerides, total cholesterol, and ApoB were all significantly higher in patients with microalbuminuria. In contrast, in the EURODIAB Study, triglycerides were the only lipid variable to remain significantly associated with
microalbuminuria after correcting for multiple confounding factors. ApoB was not measured in the EURODIAB or Estudio Diamante Studies, but in the DCCT/EDIC Study it was significantly associated with AER in a multivariate model. Hence, while dyslipidemia is more evident in advanced diabetic nephropathy, it can be seen to a lesser degree already at the stage of microalbuminuria.

7.1.3 Normoalbuminuric renal impairment

We observed that 13.4% of patients with eGFR below 60 ml/min/1.73 m² had normal AER and 2.3% of patients with normal AER had an eGFR below 60 ml/min/1.73 m² (Figure 10A and 10B). In previous studies, the frequency of normoalbuminuric renal impairment in patients with type 1 diabetes has generally varied between 8.3% and 24% (88, 343, 344). In type 2 diabetes, the most recent studies have found that 36-57% of patients with impaired renal function are normoalbuminuric (338, 345-349). The frequency of this condition has increased over the years (350), which may be explained by the use of more effective medications to treat hypertension (i.e. ACE inhibitors and ARBs) and hyperglycemia.

It has been speculated that in patients with type 2 diabetes, causes other than the classical diabetic glomerulosclerosis contribute to the development of normoalbuminuric renal impairment, e.g. ischemic vascular disease, interstitial fibrosis, or cholesterol microembolism (351). In support of this, retinopathy and albuminuria were both absent in as many as 30% of patients with type 2 diabetes and impaired renal function, indicating other causes of renal disease than true diabetic nephropathy (351). Moreover, resistance of the intrarenal arteries has been shown to be increased in patients with type 2 diabetes and impaired renal function, irrespective of albuminuria status (352). However, patients with type 1 diabetes present a more homogeneous renal phenotype, and the frequency of other causes of renal disease is substantially lower (353). Further, more advanced diabetic glomerular lesions were observed in normoalbuminuric patients with type 1 diabetes and reduced GFR (<90 ml/min/1.73 m²) than in those with normal GFR (343). Therefore, normoalbuminuric renal impairment cannot be solely explained by other causes of renal disease than diabetic nephropathy. Patients with normoalbuminuric renal impairment are more frequently women, have less retinopathy, are older, and have shorter diabetes duration than patients with albuminuric renal impairment (338, 345, 346).

The clinical significance of normoalbuminuric renal impairment has been debated, and the trait has been suggested to be explained by physiological aging. However, even if normoalbuminuric renal impairment is likely to be more benign than albuminuric renal impairment, it is still associated with a significant CVD burden (346), and both eGFR and AER have been found to be independently associated with mortality and progression to ESRD (354).
7.1.4 Achievement of recommended lipid targets

Few patients reached the internationally recommended lipid targets in our study. The targets were especially poorly met in patients with albuminuria, in patients with impaired renal function, and in patients without manifest renal disease who had poor glycemic control, were overweight, or were hypertensive. Our data suggest that many patients with type 1 diabetes are in need of lipid-lowering treatment for the prevention of CVD.

7.2 Lipid variables as predictors of progression of renal disease

Lipid abnormalities predicted progression of diabetic nephropathy at all stages of renal disease. When progression from normo- to microalbuminuria and micro- to macroalbuminuria was pooled, the overall progression of renal disease was predicted by triglycerides independently of other risk factors, including both AER and eGFR. When progression to micro- and macroalbuminuria was analyzed separately, triglycerides predicted development of incident microalbuminuria or progression to macroalbuminuria, but when AER was entered into the models triglycerides were no longer an independent predictor. The definition of renal disease may influence the results. Progression to micro- or macroalbuminuria was defined based on a change in the degree of albuminuria, and therefore, AER is already by definition a very strong predictor of progression. The power was naturally also better in the pooled analysis, especially regarding progression from micro- to macroalbuminuria, since only 50 patients progressed from micro- to macroalbuminuria during the follow-up period. ApoB also predicted progression of both micro- and macroalbuminuria in their respective analyses, but like triglycerides, not independently of AER.

In the EURODIAB Study, the triglyceride concentration was almost as strong a predictor as AER for the development of microalbuminuria, with a standardized estimate of relative risk (SERR) of 1.3 compared with 1.5 for AER (355). Regarding progression to macroalbuminuria, triglycerides were not an independent predictor of progression, but in the univariate analyses triglycerides were significantly higher in patients who progressed to macroalbuminuria and the lowest concentrations were seen in the group that regressed to normoalbuminuria (356). In the DCCT/EDIC Study, triglycerides, total cholesterol, and LDL cholesterol were all associated with progression to macroalbuminuria and regression to normoalbuminuria (357). In the Pittsburgh EDC Study, LDL cholesterol predicted development of microalbuminuria in men and in all patients combined, whereas triglycerides predicted development of microalbuminuria in women and in patients with diabetes duration of over 20 years (134). In another follow-up study of the same cohort, triglycerides, LDL cholesterol, and non-HDL cholesterol predicted progression to overt nephropathy (defined as macroalbuminuria or ESRD) within 5 years, but not in patients who progressed 6-10 years after baseline (125). In the German Diabetes Documentation System Study, triglycerides and LDL cholesterol were significant risk factors for the development of microalbuminuria. Furthermore, dyslipidemia, defined as at least one lipid
variable above the cut-off thresholds (total cholesterol >200 mg/dl ≈ 5.2 mmol/l, LDL cholesterol >160 mg/dl ≈ 4.1 mmol/l, or triglycerides >150 mg/dl ≈ 1.7 mmol/l), was associated with progression to macroalbuminuria (358).

In our study, several lipid variables predicted progression from macroalbuminuria to ESRD, e.g. high total cholesterol, triglycerides, LDL cholesterol, non-HDL cholesterol, and ApoB/ApoA-I ratio as well as low HDL cholesterol. However, when baseline eGFR was entered into the model, the only lipid variable that remained an independent predictor was total cholesterol. In a cohort from the Steno Diabetes Center including patients with type 1 diabetes and macroalbuminuria at baseline, total cholesterol was significantly associated with a decline in GFR measured with the Cr-EDTA plasma clearance technique (359). Triglycerides predicted renal failure in the World Health Organization Multinational Study of Vascular Disease in Diabetes (WHO-MSVDD) in patients with type 2 diabetes, but not in patients with type 1 diabetes (360). However, the power was much lower in patients with type 1 diabetes (only 53 vs. 134 events in patients with type 1 and type 2 diabetes, respectively), and triglycerides were only measured in a subset of the participating study centers, decreasing the power even further. In the DCCT/EDIC Study, lipid variables did not predict the development of impaired eGFR (<60 ml/min/1.73 m²) (357), however, in the Swedish National Diabetes Register (NDR) Study of patients with type 2 diabetes, triglycerides were independently associated with the development of impaired eGFR (361).

When we divided the patients into quartiles of the triglycerides, the highest quartile had the highest HR for progression of renal disease at all stages (see Figure 1 in Study II). Moreover, the hazard ratio for the development of incident microalbuminuria increased significantly with every quartile. It is noteworthy that the cut-off levels for the triglyceride quartiles were much lower than the recommended cut-off threshold of <1.7 mmol/l in the current treatment guidelines. In ROC curve analyses of triglycerides for the prediction of renal disease development and progression (see Study II Supplementary Figures 1A-C) and in Figures 12A and 12B, no clinically relevant thresholds for triglycerides with regard to the progression of renal disease emerged.

It is, however, difficult to ascertain causal links based on these data since repeated lipid and AER measurements from the day of diabetes diagnosis are not available. Whether the lipid abnormalities are primary and consistently precede the development of renal disease or are merely a consequence of renal disease has been widely debated and remains unknown. However, based on the findings from the above prospective studies, and the fact that favorable lipid profiles are associated with regression of microalbuminuria (362) and dyslipidemia with faster progression of renal disease (125, 363-366), there is an evident clinical message: dyslipidemia is associated with a poorer prognosis, especially if other risk factors, such as hyperglycemia, hypertension, smoking, and obesity, are simultaneously present.
7.3 Lipid profiles and diabetic retinopathy

In univariate analyses, multiple associations between lipid variables and PDR emerged, but in the multivariate models after correction for confounding factors, e.g. renal disease and HbA1c, only HDL cholesterol and HDL2 cholesterol were independently associated with PDR. When only patients without retinopathy or mild NPDR were included in the analyses, triglycerides and triglyceride/HDL cholesterol ratio were associated with mild NPDR independently of confounding factors. These results are in line with some of the previous studies. In the EURODIAB Study, triglycerides were independently associated with both moderate to severe NPDR and PDR (20). In the DCCT/EDIC Study, the severity of retinopathy was inversely associated with HDL cholesterol and VLDL size and positively associated with triglycerides and small- and medium-sized VLDL (367). Some earlier studies report significant associations between diabetic retinopathy and total cholesterol or LDL cholesterol (17, 368), but we only found significant associations in the univariate analyses. However, there are also several studies in which no associations between lipid variables and retinopathy status have been found (18, 19, 369).

Among prospective studies, triglycerides were independently associated with the development of retinopathy in the EURODIAB Study (370) and with the progression of retinopathy in the Pittsburgh EDC Study (371). However, lipid variables were not predictive of progression of PDR in the Sorbinil Retinopathy Trial (372) nor in the DCCT Study (373).

It is difficult to compare these studies with each other because of differing methods for detection of diabetic retinopathy, differences in the definition of retinopathy, differences in sizes of the study cohorts, and differing degree of nephropathy and availability of other risk factors for multivariate models. Relative to the strength of the associations between hyperglycemia and retinopathy and between lipid variables and CVD, the associations between lipids and retinopathy are clearly weaker, and it is impossible to draw any definite conclusions.

However, a consensus can be found regarding the association between serum lipid levels and hard exudates. Hard exudates are usually the consequence of lipid leakage from dysfunctional retinal capillaries and are considered an early sign of diabetic retinopathy and are also typically associated with maculopathy. In the WESDR Study, total cholesterol was significantly associated with the presence and severity of hard exudates (369), and in the ETDRS Study total and LDL cholesterol were also associated with hard exudates (374). In studies including mostly patients with type 2 diabetes, such as the Hoorn Study (375) and the Atherosclerosis Risk in Communities (ARIC) Study (376), associations between lipid variables and hard exudates were also confirmed.

The presence or absence of diabetic nephropathy is probably the most important confounding factor and a driving force behind the conflicting results in the studies exploring the associations between diabetic retinopathy and lipid variables. Similar
mechanisms and risk factors are thought to be behind the development of both diabetic retinopathy and nephropathy, and it is therefore no surprise that the two complications are strongly associated with each other (16). Diabetic nephropathy may lead to secondary changes in the lipid profile, and thus, it is difficult to determine whether the associations between retinopathy and the lipid variables are independent or they simply reflect the association between nephropathy and retinopathy. Therefore, multivariate models that take renal disease into account are needed, although one cannot be sure that this will eliminate the problem entirely. Notably, we found interactions between retinopathy, nephropathy, and several of the lipid variables. Due to our large cohort, we were also able to separately analyze patients with normal AER and eGFR >60 ml/min/1.73 m$^2$. Significant differences were seen in the total cholesterol/HDL cholesterol ratio by retinopathy status groups, and differences of borderline significance (p<0.05) emerged for several of the lipid variables. Clearly, associations between lipid variables and retinopathy are weaker when renal disease is taken into account, but there is also a risk for over-correction. Indeed 85% of our patients with PDR also had some signs of renal disease, and by excluding these patients from the analyses the group is no longer representative of the normal clinical setting.

To explore this dilemma from another angle, we looked at the correlations between AER and the lipid variables divided by the retinopathy status. In patients with moderate to severe NPDR or PDR, significant correlations between AER and the lipid variables were seen, as expected. However, in the patients without retinopathy, no significant correlations between AER and lipid variables were observed, and in the patients with mild NPDR only a few fairly weak correlations were present. Further, we found that in the patients without retinopathy or with only mild NPDR, AER was in fact low despite having unfavorable lipid profiles (i.e. HDL cholesterol in the lowest quartile or triglycerides, total cholesterol, or LDL cholesterol in the highest quartile). Importantly, retinopathy cannot cause secondary changes in the lipid profile so there has to be another explanation for this phenomenon. Possibly, some patients are protected from the unfavorable effects of lipid variables on microvascular complications. In contrast, the presence of severe retinopathy could serve as a marker for a more deleterious effect of hyperlipidemia and other risk factors on renal outcomes. In support of this theory, the EURODIAB Study found different associations between the blood pressure and AER in patients with and without retinopathy (377).

In light of these findings, it seems unwise to expect that the associations between lipid variables and retinopathy would be totally independent of renal disease. Furthermore, in contrast to HDL cholesterol, SBP was not independently associated with PDR when AER and eGFR were included in the multivariate model. Nevertheless, there is strong evidence for a causal relationship between blood pressure and progression of diabetic retinopathy from randomized clinical trials (165, 378).

Another problematic confounding factor is hyperglycemia. Associations exist between lipid variables and hyperglycemia, especially between triglycerides and hyperglycemia.
Again, multivariate models can take into account the confounding factors, but they cannot provide definite proof of an independent role of the factor studied. Moreover, it is not possible to draw any conclusions about a causal relationship from cross-sectional studies. Even prospective studies often have many confounding factors; hence, conclusions should be drawn with caution. Randomized clinical trials are needed to show a causal relationship. In the FIELD Trial, fenofibrate treatment reduced the need for the first laser treatment, and in the ACCORD Study patients who received fenofibrate on top of simvastatin treatment had a reduced rate of progression of diabetic retinopathy. Fenofibrate mainly reduces triglycerides and increases HDL cholesterol concentrations, however, in the FIELD Study the beneficial effect of fenofibrate did not seem to be related to any changes in the lipid concentrations. In the ACCORD Study, a clinically relevant decrease in triglycerides and increase in HDL cholesterol concentrations were seen in patients treated with fenofibrate compared with simvastatin alone, but the beneficial mechanism of fenofibrate remains unclear.

7.4 Lipid variables as predictors of a CAD event

In this study we showed that ApoB was the strongest independent predictor of an incident CAD event in the entire FinnDiane population. Triglycerides, non-HDL cholesterol, ApoB/ApoA-I ratio, and lipid ratios were also good predictors of an incident CAD event. Previous data from prospective studies in patients with type 1 diabetes are surprisingly scarce. In the EURODIAB Study, triglycerides and HDL cholesterol were independent predictors of CAD in separate models (379) after a 7-year follow-up. In the 10-year follow-up data from the Pittsburgh EDC Study, HDL cholesterol and non-HDL cholesterol predicted CAD events (380). In a Danish study, triglycerides, LDL cholesterol, and HDL cholesterol were all related to CAD after 13 years of follow-up (381). Finally, in the WHO-MSVDD Study, total cholesterol predicted the incidence of myocardial infarction in patients with type 2 diabetes, but not in patients with type 1 diabetes (382).

When men and women were analyzed separately, we found that ApoB was the strongest lipid predictor in men, whereas triglycerides, ApoB/ApoA-I ratio, and triglyceride/HDL cholesterol ratio had the highest HR per SD increase in women (see Study IV Supplementary Table 4). In the EURODIAB Study, triglycerides also predicted CAD in women. However, in that study, apolipoproteins were not measured and lipid ratios were not calculated.

The predictive performance of LDL cholesterol in this study was poor. In the entire cohort, the NRI for LDL cholesterol was 3.2%, compared with 7.7% for ApoB. Moreover, in patients without renal disease, LDL cholesterol was not an independent predictor of CAD, and Figure 1A (in Study IV) shows that the median LDL cholesterol level was not able to separate cases from controls. Further, in patients with HbA1c below 8.3% and in women, LDL cholesterol could not predict CAD independently of other risk factors.
Experimental studies have shown that remnant cholesterol accumulates in the arterial wall like LDL cholesterol (383, 384), and importantly, both ApoB and non-HDL cholesterol are capturing the risk of all the atherogenic particles, including the VLDL, IDL, and LDL. Of note, the mean baseline LDL cholesterol concentration (3.45 mmol/l) of patients with an incident CAD event in this study was lower than the mean LDL cholesterol concentrations in older clinical trials of lipid-lowering treatment (385). In addition, the presence of sdLDL particles is not revealed by LDL cholesterol concentrations. ApoB is a better detector of an increased number of sdLDL particles (see also Section 2.3.2), and in concordance, ApoB was a stronger predictor of CAD than LDL cholesterol in our study. Furthermore, already in children with type 1 diabetes a preponderance of sdLDL compared with children without diabetes has been reported (238). There are also several limitations regarding the LDL cholesterol calculated by the Friedewald formula. The best known is the underestimation of LDL cholesterol in hypertriglyceridemic conditions. LDL cholesterol should not be calculated using this formula in patients with triglyceride concentrations above 4.0 mmol/l, but as a consequence, several high-risk patients (N=48) could not be included in the multivariate models with LDL cholesterol as the lipid variable. Concerns have also been raised about the accuracy of the Friedewald LDL cholesterol at much lower triglyceride concentrations (386, 387) it has been found to underestimate LDL cholesterol concentrations already when triglycerides exceeds 1.7 mmol/l (388). Of note, also in previous prospective studies in patients with type 1 diabetes, other lipid variables have emerged as better predictors of CAD events than LDL cholesterol (379, 380). Data from randomized clinical trials have established LDL cholesterol lowering as the primary target of therapy for prevention of CAD, but the residual risk present beyond that of LDL cholesterol should be recognized in clinical practice, especially in patients with type 1 diabetes with fairly good glycemic control, in patients without renal disease, and in women.

In this study, HDL cholesterol was a weaker predictor of CAD than atherogenic lipid variables. Its best performance was seen in patients with normal AER at baseline; however, also in these patients, triglycerides and ApoA-I were stronger predictors of CAD. The relationship between HDL cholesterol and CAD is far more complex than initially assumed. Despite the strong inverse correlation between HDL cholesterol and CAD seen in epidemiological studies, a pharmacological increase of HDL cholesterol has failed to reduce the risk of CAD. Further, a Mendelian randomization study showed that a genetic mechanism that raised HDL cholesterol did not lower the risk of myocardial infarction, thereby calling into question the assumed causal relationship between low HDL cholesterol concentrations and CAD risk (389). From clinical practice, we know that low HDL cholesterol is rarely an isolated trait and is most often accompanied by increased triglycerides, abdominal obesity, and other components of metabolic syndrome. Therefore, we may have jumped to a conclusion by assuming a causal relationship between reduced HDL cholesterol and CAD risk. The poorer performance of HDL cholesterol compared with atherogenic variables could also be related to changes in functionality and a possible loss of protective effects of HDL cholesterol in patients with type 1 diabetes. The possible
functionality changes cannot be captured by the mere measurement of HDL cholesterol concentrations or lipid ratios, but interestingly, the lipid and lipoprotein ratios containing atherogenic and anti-atherogenic particles were among the strongest predictors of CAD in women and in healthier patients (i.e. patients without renal disease or with HbA1c below 8.3%). By contrast, in patients with renal disease, in patients with HbA1c above 8.3%, or in men, no additional benefit was gained from the ratios compared with the atherogenic lipid variables alone.

The frequency of patients with a history of smoking was high in the cohort, but the frequency of current smoking at baseline was much lower. Current smoking was not univariately associated with CAD events, whereas history of smoking was, and therefore history of smoking was chosen for the multivariate models. However, history of smoking was not an independent predictor of CAD, whereas several lipid variables were. Therefore, in this study smoking did not explain the high CAD risk any better than did lipid variables; however, both are definitely important risk factors that should be treated aggressively and are likely to have a potentiating effect. A dual increase in chronic inflammation could, for example, be one of the driving mechanisms for a potentiating effect.

When we performed a Cox regression analysis with clustering of risk factors as the independent variable, we could see that the rise in HR was not linear. For patients with no more than two of any of the five risk factors, the HR was not significantly different from that of the reference group. However, in patients with three risk factors the HR was already 3.27, in patients with four risk factors the HR was 5.96, and in those with all five risk factors the HR was 7.02 compared with the reference group. Our results indicate a potentiating effect with an increasing number of risk factors in patients with three or more risk factors.

The metabolic syndrome is a cluster of risk factors dominated by central obesity, increased triglycerides, decreased HDL cholesterol, and elevated blood pressure. In this study, eGDR, a formula for insulin sensitivity that includes WHR, HbA1c, and hypertension, was clearly lower in patients with CAD than in controls (4.49 vs. 6.90 mg/kg/min). In the Pittsburgh EDC cohort, eGDR was shown to predict lower extremity arterial disease (390), macroalbuminuria (125), and hard CAD events (380).

### 7.5 Strengths and limitations

The patients in Studies I, II, and IV account for roughly 10% of all patients with type 1 diabetes in Finland and show even distribution geographically, closely following the distribution of the general population in Finland (see Figure 8). A selection bias is therefore less likely than in single hospital-based studies. Furthermore, the high response rate of 78% (325) makes any significant selection biases unlikely. An additional strength is that all lipid variables were measured in the same laboratory specializing in lipid
research and that the phenotypes for diabetic nephropathy, retinopathy, and CAD were robustly and meticulously assessed. Thus, this large cohort is unique for the detailed study of lipid profiles of patients with type 1 diabetes.

A limitation of Studies I and III was the cross-sectional study design; however, Study I was followed by a subsequent prospective study. In Study III, the retinopathy status was determined in 1465 consecutively recruited patients with complete lipid profiles. To avoid a selection bias, the patients were derived from the first patients participating in the FinnDiane Study. In Study II, the progression of renal disease was ascertained after a review of the medical files, and therefore, the follow-up information was not dependent on patients’ participation in a revisit, only on their regular visits to their physician. In Study IV, the follow-up information on CAD events was obtained from the Finnish Hospital Discharge Register as well as the Causes of Death Register; hence, follow-up information was available for all patients. A limitation of the studies is the possible survival bias, however, this was accounted for by performing competing risk regression analyses in Study IV.

Another limitation of the study is that it is not feasible to measure the GFR directly in such a large cohort. In Study I, we used the Cockcroft-Gault formula (71). This formula was developed in a population of hospitalized men with a fairly wide range of renal function. Because the estimate also includes the tubular secretion of creatinine, it has been found to systematically overestimate the actual renal function (391). In Study II, we used both the Cockcroft-Gault and the MDRD-4 equation (72). The MDRD equation was developed in patients with chronic kidney disease. It has been validated in Caucasian populations aged between 18 and 70 years with eGFR <60 ml/min/1.73 m² and has shown good performance for patients with impaired renal function. However, the MDRD equation has been found to be less accurate in populations without impaired renal function (392, 393). The CKD-EPI equation (73) is the newest of the eGFR equations and was used in Study IV. It was developed in a population with a wide range of measured GFR to address the issue of underestimation of eGFR by the MDRD equation at eGFR levels above 60 ml/min/1.73 m². The CKD-EPI equation has been found to be as accurate as the MDRD equation when GFR is <60 ml/min/1.73 m² and more accurate when GFR is between 60 and 120 ml/min/1.73 m² (73).

Unfortunately, renal biopsies were not available from our patients. However, in patients with type 1 diabetes, the renal phenotype is much more homogeneous than in patients with type 2 diabetes (353) as previously discussed in Section 7.1.3. The presence of retinopathy is also considered to support the diagnosis of diabetic nephropathy (394). In our patients with ESRD, 98.5% had a history of laser treatment, which is performed to treat PDR or severe NDPR in the vast majority of patients (161).
7.6 Lipid variables and micro- and macrovascular complications

Triglycerides were associated with AER, eGFR, and mild NPDR and predicted the progression of renal disease at all stages of albuminuria and incident CAD events in the total population, in women, and in patients with normal AER. Triglyceride concentrations have been shown to be strongly related to glycemic control (337), and it is widely known that corrections of severe hyperglycemia will also lead to lower triglycerides. However, in all four studies, triglycerides were associated with or predicted adverse events independently of HbA1c. Furthermore, triglycerides were not the only lipid variable associated with adverse microvascular events, for example, ApoB also predicted progression to micro- and macroalbuminuria, total cholesterol predicted progression to ESRD, and HDL cholesterol was associated with PDR. Therefore, our results support an additive effect of triglycerides and other lipid variables on microvascular complications beyond glycemic control.

Triglycerides are often a univariate predictor of CVD, but not an independent predictor when the multivariate models are adjusted with other lipid variables, which is probably due to the strong correlations between triglycerides and both non-HDL cholesterol and HDL cholesterol (335). A study from the Emerging Risk Factor Collaboration (ERFC) including 68 prospective studies, with individual data from a total of 302 430 participants, showed that triglycerides were not an independent predictor of CAD after adjustments for HDL and non-HDL cholesterol, whereas HDL cholesterol and non-HDL cholesterol remained independent predictors after the adjustments (395). In addition, there is no evidence from clinical trials that lowering triglycerides reduces CVD events after adjusting for HDL cholesterol. However, a genetic association study showed that triglycerides are a causal risk factor of CAD independently of HDL and LDL cholesterol, whereas genetic variants primarily associated with HDL cholesterol were not associated with CAD after adjustments with triglycerides and LDL cholesterol (396) (see also discussion in Section 7.4). Importantly, increased triglyceride concentrations are associated with higher remnant cholesterol concentrations (i.e. VLDL and IDL cholesterol as well as chylomicron remnants in the non-fasting state). A causal association between increased remnant cholesterol concentrations and both CAD and low-grade inflammation was shown in a Mendelian randomization study, whereas increased LDL cholesterol concentrations were causally associated with CAD, but not with inflammation (397). LDL cholesterol will remain the primary treatment target due to strong evidence from clinical trials, but increasing evidence highlights the superiority of either non-HDL cholesterol or ApoB over LDL cholesterol for CVD risk prediction (398-400). However, expert opinion is divided regarding whether or not apolipoprotein measurements should replace cholesterol measurements, and the evidence is conflicting. The ERFC Study found that non-HDL/HDL cholesterol and ApoB/ApoA-I ratios had very similar HR for CAD (395), whereas in a meta-analysis including 233 455 participants the mean relative risk ratio for ApoB was 12% higher than for LDL cholesterol and 6% higher than for non-HDL cholesterol (401). The conflicting results could be caused by differences between study populations and in the proportions of patients with metabolic dyslipidemia (i.e. high
triglycerides, low HDL cholesterol, and sdLDL cholesterol) included in the studies, and it has been suggested that ApoB may be a better CVD predictor in these patients (402). In our study, the HR of ApoB and non-HDL cholesterol for CAD events was 1.40 and 1.27, respectively, and in the NRI analyses ApoB correctly reclassified more patients than non-HDL cholesterol when it was added to the multivariate model (7.7% [p=0.01] vs. 4.7% [p=0.06]). All in all, the difference between non-HDL cholesterol and ApoB seems to be small at least in the general population, and the clear benefits of calculating non-HDL cholesterol lie in its good clinical availability and in creation of no additional costs when total cholesterol and HDL cholesterol have been measured. On the other hand, ApoB measurements have become less expensive, their clinical availability has improved, and computationally estimated ApoB with strong correlations (r=0.93-0.98) and no additional costs has been developed (403). As both non-HDL cholesterol and ApoB perform better than LDL cholesterol, the use of either one in the clinical setting should be emphasized in order to capture residual CAD risk.

7.7 Lipid profiles in patients with “double diabetes”

Importantly, in patients with renal disease, poor glycemic control, or high BMI, as seen in Study I, the lipid profile resembles that of patients with type 2 diabetes. We have previously shown that the weight-adjusted insulin dose tends to be similar or even higher in such patients (404), which suggests that the dyslipidemia is more related to increased insulin resistance than to inadequate insulin administration. The concept of “double diabetes” (i.e. when patients with type 1 diabetes exhibit features of type 2 diabetes and insulin resistance) has been proposed to describe this phenomenon (405). The prevalence of double diabetes is increasing as a consequence of increased adiposity worldwide, and the prevalence of metabolic syndrome in the Finndiane cohort is as high as 40% in women and 38% in men (230). The triglyceride/HDL cholesterol ratio, which strongly correlates with insulin resistance (406), predicted progression to macroalbuminuria and ESRD in Study II, was associated with retinopathy in Study III, and predicted CAD events in the entire cohort, in women, in patients with normal AER, and in patients with HbA1c below the median of the cohort in Study IV. These data are supported by the DCCT Study, in which eGDR, an estimate of insulin sensitivity, strongly predicted the development of nephropathy, retinopathy, and CVD (232).

7.8 Lipid thresholds and prediction

Cut-off values are widely used in the clinical setting, however, many continuous biological risk factors lack clear thresholds. This is also true for the lipid variables. In our study we could not find any clinically relevant thresholds for any of the lipid variables with regard to either micro- or macrovascular complications. However, the currently recommended cut-off level (≤1.7 mmol/l) for triglycerides seems to be too high for
patients with type 1 diabetes with regard to renal disease progression and prediction of an incident CAD event. However, the cut-off of 1.7 mmol/l derives from studies for the prevention of cardiovascular disease in the general population. Thus, the cut-off for prediction of renal disease progression is unknown, but it is also noteworthy that the currently recommended cut-off level was unable to predict an incident CAD event in patients with normal AER. AER is also a continuous risk factor for micro- and macrovascular complications, and even a mild increase within the normoalbuminuric range predicts adverse outcomes (407, 408). Therefore, it has been proposed that we should cease to use the traditional categories of normo-, micro-, and macroalbuminuria and aim for an earlier risk evaluation by a multifactorial approach. Studies of renal biopsies from patients with type 1 and type 2 diabetes also support this notion (353).

7.9 Multifactorial approach

Multiple risk factors often cluster in the same patients. In Study IV, we showed that the increase in HR for incident CAD events was not linear, and an additive effect with increasing number of risk factors in patients with three or more risk factors was seen. From a practical point of view, risk calculators are needed for clinicians to be able to take into account multiple and continuous risk factors. Many calculators already exist for the prediction of CVD, but few are specifically designed for patients with type 1 diabetes, and risk calculators for prediction of renal disease are even scarcer. A multifactorial approach is also needed for the prevention of both micro- and macrovascular complications. In the Steno-2 Study, including patients with type 2 diabetes and microalbuminuria at baseline, 160 patients were randomized to receive either conventional or intensified multifactorial treatment (188). Intensified treatment included both lifestyle modifications and pharmacological treatments and reduced, for example, total cholesterol by 50 mg/dl ≈ 1.3 mmol/l, triglycerides by 41 mg/dl ≈ 0.5 mmol/l, SBP by 14 mmHg, and HbA1c by 0.5%. After 7.8 years of follow-up, intensified treatment reduced the risk of CVD events by 53%, progression to macroalbuminuria by 61%, and development or progression of retinopathy by 58%. Experimental studies also support a multifactorial pharmacological approach. In rats with massive proteinuria and renal lesions, the combination of an ACE inhibitor and a statin significantly reduced glomerulosclerosis, interstitial inflammation, and tubular damage, more than the effect of either drug alone (318). However, it is quite clear that more randomized clinical trials are still needed to clarify the role of lipid variables and lipid-lowering treatment in the prevention of microvascular complications. We still lack large trials with multifactorial approaches that are initiated at an early stage of the disease process, take into account concomitant microvascular complications, and include a sufficient number of patients and sufficiently long follow-up periods.
8 SUMMARY AND CONCLUSIONS

8.1 Study I

In patients with type 1 diabetes, not only increased AER but also impaired renal function (eGFR<60 ml/min/1.73 m²) is associated with lipid abnormalities. Changes in the lipid profile can be seen already at the stage of microalbuminuria, but are more evident in patients with macroalbuminuria. A number of patients in this study would have exceeded the internationally recommended lipid targets for the prevention of CVD, and the targets were particularly poorly met with respect to the LDL cholesterol concentrations. In patients with type 1 diabetes and impaired renal function or macroalbuminuria, the targets were especially poorly met, even though these are the patients who should be treated most aggressively. Further, patients without signs of renal disease, but with poor glycemic control, hypertension, or obesity also frequently exceeded the recommended lipid targets.

8.2 Study II

Triglycerides were an independent risk factor for the development or progression of renal disease at all stages. ApoB was also an independent predictor of progression to micro- and macroalbuminuria. Total cholesterol predicted progression from macroalbuminuria to ESRD independently of eGFR. The triglyceride concentration needed to increase the risk of progression of renal disease was much lower than the currently recommended cut-off level for triglycerides (<1.7 mmol/l), which is based on studies aimed at preventing cardiovascular disease. When ROC curves and predicted probability plots were performed, no clinically relevant thresholds emerged, but whether lower lipid targets than those currently recommended for CVD would be beneficial with regard to the progression of renal disease remains to be elucidated.

8.3 Study III

The total HDL and HDL₂ cholesterol concentrations were inversely associated with PDR independently of diabetes duration, metabolic control, blood pressure, and renal disease. The triglycerides were independently associated with mild NPDR. We observed interactions between retinopathy, nephropathy, and most lipid variables. The previously reported associations between AER and lipid variables were not seen in patients without signs of retinopathy. Furthermore, the correlations between AER and lipid variables were much stronger in patients with PDR than in patients with only mild NPDR. The results were also replicated in an independent cohort of 1100 patients with information on laser
treatment available. The results suggest the existence of shared pathological mechanisms between diabetic retinopathy and nephropathy.

8.4 Study IV

The predictive ability of lipid variables differed substantially depending on the patient’s sex, renal status, and glycemic control and was substantially different from the traditional cholesterol-centric view. Total and LDL cholesterol were poor predictors of an incident CAD event in patients with normal AER, in patients with HbA$_{1c}$ below the median of the cohort, and in women, in whom the ratios of atherogenic and anti-atherogenic lipoproteins and lipids as well as triglycerides performed better. The current guidelines may need to be revised to capture residual CAD risk in patients with type 1 diabetes.
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Helsinki, December 2014

Nina Tolonen
### APPENDIX

<table>
<thead>
<tr>
<th>The Finnish Diabetic Nephropathy Study Centers</th>
<th>Physicians and nurses</th>
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<tr>
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