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Long term complications in juvenile diabetes mellitus

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To my family
To all children with diabetes

Abbreviations

ADA	The American Diabetes Association
AER	Albumin excretion rate
BMI	Body mass index
BMT	Basal membrane thickness
CI	Confidence interval
CV	Coefficient of variance
CVD	Cardiovascular disease
DCCT	Diabetes Control and Complication Trial
DKA	Diabetic ketoacidosis
ESRD	End stage renal disease
GFR	Glomerular filtration rate
GH	Growth hormone
HbA_{1c}	Glycated haemoglobin A ₁
IGF-1	Insulin-like growth factor 1
OR	Odds ratio
RR	Relative risk
SD	Standard deviation
SMR	Standardized mortality ratio
VPT	Vibration perception threshold

Contents

Abstract	1
Swedish summary / Svensk sammanfattning	3
Original publications	5
Background	7
Classification of diabetes mellitus.....	7
Pathogenesis of Type 1 diabetes mellitus	7
Epidemiology	8
Late complications	8
History.....	8
Classification.....	9
Macrovascular complications.....	9
Microvascular complications	10
Diabetic nephropathy	10
Diabetic retinopathy	12
Diabetic neuropathy	13
Risk factors.....	16
C-peptide	20
Mortality.....	22
Ethical considerations	23
Aims of the study	25
Methods	27
Study population	27
Design	30
Definitions and laboratory methods	31
Retinopathy	31
Nephropathy	31
Neuropathy	31
Metabolic control	32
Cardiovascular risk factors.....	33
C-peptide secretion.....	33
Partial remission.....	34
Diabetic ketoacidosis.....	34
Statistical analysis	34
Results	35
Incidence of diabetic retinopathy and nephropathy	35
Risk factors for retinopathy and nephropathy.....	38
Metabolic control	39
Diabetic complications in patients with intensive treatment from onset of diabetes	41
Clinical characteristics of patients at onset of diabetes during 25 years	42
Secular trend of diabetes partial remission and C-peptide secretion	42
C-peptide secretion and metabolic control.....	45
Mortality.....	47
Discussion	49
Study population	49
Statistical methods.....	49
Definitions of long term complications.....	50
Laboratory methods.....	51

Incidence and prevalence of long term complications	51
Risk factors.....	53
Clinical picture at diagnosis	55
C-peptide secretion and partial remission	56
Conclusion.....	57
Future research	58
Acknowledgements.....	59
References	61
Papers I - IV	

Abstract

Background/aim. The incidence of microvascular complications has been reported to be unchanged the last decades. However, in randomized clinical trials it has been shown that improved metabolic control can reduce the development of long term complications. It has been debated whether it is possible to achieve the same results in an unselected population. In a previous study we found a decreased incidence of overt nephropathy, but unchanged incidence of severe laser treated retinopathy in a population of patients with Type 1 diabetes diagnosed in childhood. The aim of the present study was to investigate the incidence 10 years later in the same population and to analyse the importance of possible risk factors. In another previous study we found a high prevalence of subclinical neuropathy among young diabetic patients despite intensive insulin therapy since diagnosis. The aim of the present study was to examine if intensive treatment is more effective in preventing early diabetic complications other than neuropathy. The incidence of Type 1 diabetes has doubled in Sweden the last decades. The reason must be environmental factors. These, as well as more intensive insulin regimens from onset of diabetes, might also lead to different disease process. We wanted to analyse if clinical characteristics at onset had changed the last 25 years and if there was any secular trend of C-peptide secretion. We also intended to investigate if longer persistence of C-peptide secretion could be of importance for prevention of long term complications.

Methods. The whole study population consisted of all 478 patients with Type 1 diabetes diagnosed before the age of 15 during the years 1961 – 2000, living in the catchment area of the Paediatric Clinic, University Hospital, Linköping, Sweden. For the statistical analysis the population was divided into five-year cohorts according to time of onset of diabetes. The cumulative proportion of severe retinopathy and overt nephropathy in 269 patients with onset of diabetes 1961 – 1985 was computed with survival analysis. Multivariable regression models were used to analyse the importance of metabolic control, diabetes duration, blood pressure, smoking, BMI, lipids and persisting C-peptide secretion. The prevalence of all grades of retinal changes, nephropathy and neuropathy, defined as abnormal nerve conduction, was estimated in the late 1990s in a subgroup of 80 children and adolescents with mean 13 years of diabetes duration. Clinical characteristics at onset, duration of partial remission and regularly measurements of fasting and stimulated C-peptide secretion the first five years after onset were analysed in 316 patients with onset of diabetes 1976 – 2000.

Results. The cumulative proportion of severe laser treated retinopathy showed a significant declining trend the last decades. The decrease was significant between the oldest cohort with diabetes onset 1961 – 1965 and the cohorts with diabetes onset 1971 – 1975 and 1976 – 1980. The cumulative proportion of overt nephropathy also declined with a significant decrease between the oldest cohorts and all the following cohorts. After 25 years of diabetes duration it was 30% and 8% in the two oldest cohorts respectively and remained largely unchanged after 30 years. Diabetes duration and long term HbA_{1c} were the only significant independent risk factors for both retinopathy and nephropathy. The risk of overt nephropathy increased substantially when HbA_{1c} was above 8.5%, while the risk of severe retinopathy increased already when HbA_{1c} exceeded 7.5%. The prevalence of neuropathy was 59%, of retinopathy 27% and of nephropathy 5% in the population of young patients after 13 years of diabetes duration. During the last 25 years the clinical characteristics at onset were unchanged as well as duration of partial remission and magnitude and persistence of C-peptide secretion.

Conclusions. In this unselected population the cumulative proportion of severe retinopathy and overt nephropathy decreased over the last decades. Diabetic nephropathy has probably been prevented and not just postponed. Good glycaemic control was the most important factor to avoid complications, with the necessity of a lower level of HbA_{1c} to escape retinopathy than nephropathy. Intensive insulin regimens from diabetes onset was not sufficient to entirely escape early diabetic complications after mean 13 years of diabetes duration, even if the prevalence of retinopathy and especially nephropathy was lower than usually reported. The clinical picture at onset of diabetes was unchanged the last 25 years. There was no secular trend of partial diabetes remission or C-peptide secretion during the first years after diagnosis.

Swedish summary / Svensk sammanfattning

Bakgrund: Diabetes mellitus typ 1 är en allvarlig kronisk sjukdom, som utan behandling med insulin är livshotande. Orsaken till diabetes är okänd, men man misstänker att olika miljöfaktorer, t ex virusinfektioner, i kombination med en ärftlig känslighet leder till en autoimmun förstörelse av de insulinproducerande betacellerna i bukspottkörteln. Trots insulinbehandling har man hittills inte kunnat undvika utvecklandet av svåra långtidskomplikationer. Dessa debuterar efter 10 – 20 års diabetes och brukar indelas i skador på de små blodkärlen i ögon (retinopati), njurar (nefropati) eller nerver (neuropati) och skador på de stora blodkärlen, som kan leda till hjärtinfarkt, stroke eller andra hjärtkärlsjukdomar. Trots modern behandling har man i många studier de senaste decennierna inte kunnat visa någon minskad risk för njurskador, som drabbade 30 – 40 % av patienterna och är den vanligaste orsaken till njursvikt med behov av dialys eller njurtransplantation. Svåra ögonskador drabbade mer än 60 % efter 35 års diabetes och är fortfarande den vanligaste orsaken till blindhet i västvärlden. Dödligheten är förhöjd, framför allt hos de patienter som drabbas av njurskador. Flera interventionsstudier har övertygande visat betydelsen av god blodsockerkontroll för att undvika långtidskomplikationer, men det måste finnas även andra förklaringar. En del patienter drabbas trots god blodsockerkontroll och omvänt kan en del patienter undslippa komplikationer, åtminstone njurskada, trots dålig blodsockerbalans. Högt blodtryck och andra riskfaktorer i det s.k. metabola syndromet har misstänkts ha betydelse liksom rökning och ärftliga faktorer. Resultatet från studier av olika populationer är dock motsägelserfulla. Intresset har de senaste åren ånyo riktats mot C-peptid, som vid sin upptäckt på 1960-talet inte ansågs ha någon egen biologisk effekt. C-peptid är en del av proinsulinmolekylen, som bildas som ett förstadium till insulin i betacellerna i bukspottkörteln. C-peptiddelen klyvs av och utsöndras till blodbanan i samma mängd som insulin och kan användas som ett mått på den egna kvarvarande insulinproduktionen. Studier från bl a Linköping har visat att förvånansvärt många barn har kvar egen insulinproduktion vid insjuknandet i diabetes. Andra studier från senare år har visat att C-peptid sannolikt har en egen effekt, som möjligen skulle kunna bidra till att minska förekomsten av långtidskomplikationer.

I en tidigare studie från Linköpingsregionen visades en glädjande minskning av njurkomplikationer de senaste decennierna, men oförändrad risk att drabbas av ögonskador. I en annan studie påvisades en hög förekomst av nervpåverkan hos unga vuxna efter bara i medeltal 13 års diabetes och med modern insulinbehandling redan från debuten av diabetes.

Syfte: Syftet med de aktuella studierna har varit att ytterligare studera hur vanligt långtidskomplikationer är i en väl definierad oselektad population efter lång tids uppföljning och att närmare analysera betydelsen av olika riskfaktorer, som skulle kunna förklara den förbättrade långtidsprognosen. Vi ville också studera om intensiv insulinbehandling bättre kan förebygga andra tidiga tecken på långtidskomplikationer än nervskador. I ytterligare en studie undersöktes om diabetessjukdomens svårighetsgrad vid debuten förändrats de senaste 25 åren och om förekomsten av kvarvarande C-peptidsekretion ökat, vilket skulle kunna tänkas bidra till den observerade minskningen av långtidskomplikationer.

Metod: Hela studiepopulationen utgjordes av alla de barn, som insjuknade i typ 1 diabetes före 15 års ålder under tidsperioden 1961 – 2000 och som då var bosatta i Linköpingsregionen. De indelades i 5-årsgrupper (kohorter) beroende på insjuknandeår. Andelen som drabbats av svår laserbehandlad ögonskada och svår njurskada fram till slutet av 1990-talet beräknades med s.k. överlevnadsanalys för de 269 patienter som insjuknat i diabetes mellan åren 1961 – 1985. En jämförelse gjordes mellan de olika kohorterna. Betydelsen av diabetessjukdomens varaktighet (duration), blodsockerbalans (mätt som långtidsHbA_{1c}), långtidsblodtryck, övervikt, rökning, blodfetter, ärftliga faktorer och förekomst av kvarvarande C-peptidsekretion för utvecklande av ögon- och njurskador analyserades. Bortfallet i studien var lågt och 91 –95 % kunde följas till åtminstone 1997.

Förekomsten av alla grader av ögonförändringar, njurpåverkan och nervpåverkan undersöktes hos 80 unga vuxna, som i slutet av 1990-talet haft diabetes i medeltal 13 år. Dessa patienter var behandlade med intensiv flerdosbehandling med insulin redan från debuten av diabetes.

Den kliniska bilden vid insjuknandet undersöktes hos alla de 316 barn, som insjuknade i diabetes under åren 1976–2000. Typ av insulinterapi vid debuten och de närmaste åren därefter registrerades liksom förekomst och varaktighet av s.k. partiell remission (period med lågt insulinbehov). C-peptidsekretionen vid debuten och därefter årligen analyserades så länge som barnen kontrollerades på barnkliniken. Vid 5 tillfällen under de första 4 åren bestämdes också C-peptid efter stimulering med standardiserad frukost.

Resultat: Andelen patienter med svåra ögonskador minskade de senaste decennierna. Efter 25 års diabetes hade den sjunkit från 47 % för de patienter, som insjuknat i diabetes 1961–65 till 28 % respektive 24 % för dem som insjuknat 1966 – 70 och 1971 – 75. Efter 30 års diabetes hade den stigit till 53 % för den äldsta kohorten och till 44 % för de som insjuknat 1966 – 70. Skillnaden var statistisk signifikant mellan den äldsta kohorten med debut 1961 – 65 och kohorterna med debut 1971 – 75 och 1976 – 80. Förekomsten av lindrigare ögonskador var oförändrat hög i alla grupperna. Andelen patienter med svår njurskada minskade också och skillnaden var signifikant mellan de som fått diabetes 1961 – 65 och alla de efterföljande kohorterna. Efter 25 års diabetes var den 30 % för dem som insjuknat 1961 – 65 och sjönk till 8 % respektive 13 % för dem som insjuknat 1966 – 70 och 1971 – 75. Andelen var i stort sett oförändrad efter 30 års diabetes, 32 % respektive 11 % i de äldsta kohorterna. Förekomsten av lindrigare njurpåverkan var oförändrat låg. Patienterna med ögon- och njurkomplikationer hade haft diabetes längre, hade högre HbA_{1c}, högre blodfetter, rökte oftare och hade oftare hjärtkärlsjukdomar och högre blodtryck än patienter utan komplikationer. I multivariabla statistiska modeller kvarstod emellertid enbart diabetesduration och HbA_{1c} som signifikanta riskfaktorer. Risken för svåra ögonkomplikationer ökade påtagligt redan vid HbA_{1c} > 7,5 %, medan risken för svåra njurskador ökade först när HbA_{1c} översteg 8,5 %.

Förekomsten av nervpåverkan var 59 %, ögonskada 27 % och njurskada endast 5 % i gruppen av unga vuxna med diabetes. Endast 30 % hade inga tecken på komplikationer alls. Det fanns endast enstaka fall av svårare former av långtidskomplikationer. Blodsockerkontrollen var, jämfört med andra studier, relativt god med medelHbA_{1c} 7,3 %. HbA_{1c} var högre för de patienter, som hade nerv- och ögonpåverkan.

Den kliniska svårigheten av diabetes vid insjuknandet har varit oförändrad de senaste 25 åren. Insjuknandet i diabetes har fördubblats och insulinbehandlingen har blivit mer intensiv med intravenöst insulin vid insjuknandet och flerdosbehandling med insulin redan från debuten som standard. C-peptidsekretionen under de första åren och förekomst och varaktighet av partiell remission var också oförändrad.

Slutsatser: Andelen patienter med svåra njur- och ögonskador har minskat påtagligt de senaste decennierna. Denna minskning har varit möjlig i en oselektad population. Detta har inte tidigare visats vad gäller ögonskador. Njurskadorna verkar ha förhindrats, medan vi ännu inte vet om vi kan förhindra eller bara skjtit upp ögonskadorna. God blodsockerbalans var den absolut viktigaste faktorn för att undvika långtidskomplikationer. Det krävs bättre blodsockerkontroll med lägre HbA_{1c} för att förhindra ögonskador än njurskador. Intensiv insulinterapi redan från debuten och relativt god blodsockerbalans räckte inte för att undvika tidiga lindrigare former av långtidskomplikationer. Nervpåverkan var vanlig, ögonpåverkan verkar ha minskat medan förekomsten av njurpåverkan var låg jämfört med äldre studier. Den kliniska svårigheten vid insjuknandet har varit oförändrad de senaste 25 åren, trots att insjuknandefrekvensen fördubblats. C-peptidsekretionen under de första åren och förekomst och varaktighet av partiell remission var också oförändrad trots att insulinbehandlingen blivit mer intensiv. Förekomst av C-peptidsekretion de första fem åren kan inte förhindra utvecklandet av långtidskomplikationer.

Original publications

The thesis is based on the following papers, which will be referred to in the text by their roman numerals.

I

Nordwall M., Bojestig M., Arnqvist H., Ludvigsson J.
Declining incidence of severe retinopathy and persisting decrease of nephropathy in an unselected population of Type 1 diabetes-the Linköping Diabetes Complications Study. *Diabetologia*, 2004. 47(7): 1266 – 72.

II

Nordwall M., Hyllienmark L., Ludvigsson J.
Early diabetic complications in a population of young patients with Type 1 diabetes mellitus despite intensive treatment. *J Pediatr Endocrinol & Metab*, 2006. 19(1): 45 – 54.

III

Nordwall M., Ludvigsson J.
Unchanged clinical picture and beta cell function of diabetic children in spite of doubling incidence and different treatment the last 25 years.
Manuscript

IV

Nordwall M., Bojestig M., Arnqvist H., Ludvigsson J.
Good metabolic control remains crucial in prevention of late diabetic complications-the Linköping Diabetes Complications Study.
Manuscript

Background

Classification of diabetes mellitus

Diabetes mellitus is a syndrome characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. An actual etiologic classification suggested by the American Diabetes Association and based on our other present knowledge is presented in Table 1¹. Type 1 diabetes only accounts for 5 – 10% of those with diabetes, but is the most predominant form of diabetes diagnosed in childhood. Type 2 diabetes is strongly associated with obesity. In the United States the increasing rate of obesity the last decades has resulted in an increasing incidence of Type 2 diabetes even in adolescents, especially in some ethnic groups as Hispanics and blacks². The incidence of obesity is also increasing in Sweden, although not to the same degree as in other parts of the western world^{3, 4}. However, until today there are no reports of increasing rate of Type 2 diabetes among young adults in Sweden⁵. This study has focused on Type 1 diabetes diagnosed before the age of 15 years.

Pathogenesis of Type 1 diabetes mellitus

The combination of genetic susceptibility and environmental factors is proposed to lead to an autoimmune cellular and humoral mediated destruction of the beta cells in the pancreas. There is a strong association between certain HLA genes and risk for Type 1 diabetes, but the frequency of the high risk genotypes differs among children of different ages⁶. Children with newly diagnosed diabetes have autoantibodies against glutamic acid decarboxylase (GADA), islet cells (ICA), insulin (IAA) or tyrosine phosphatase (IA - 2) in 85 – 98%, but the rate of antibody positivity varies among different age groups^{6,7}.

There is a rather low concordance for diabetes of about 40% between monozygotic twins⁸⁻¹⁰. The rapidly increasing incidence of Type 1 diabetes the last decades cannot be explained by genetic changes in this short time perspective¹¹. These factors speak in favour of the importance of environmental factors for the development of Type 1 diabetes. In epidemiological studies several environmental factors has been associated with diabetes.

Table 1 Etiologic classification of diabetes mellitus

I	Type 1 diabetes mellitus (beta cell destruction, leading to insulin deficiency)
	a. Immune mediated
	b. Idiopathic
II	Type 2 diabetes mellitus (different grades of insulin resistance with different grades of relative insulin deficiency)
III	Other specific types
	a. Genetic defects of beta cell function (e.g MODY Maturity Onset Diabetes of the Young)
	b. Genetic defects in insulin action
	c. Diseases of exocrine pancreas (e.g. pancreatitis, cystic fibrosis)
	d. Endocrinopathies (e.g. Cushing's syndrome)
	e. Drug- or chemical- induced (e.g. corticosteroids)
	f. Infections (e.g. congenital rubella)
	g. Uncommon form of immune-mediated diabetes mellitus
	h. Genetic syndromes associated with diabetes mellitus
IV	Gestational diabetes mellitus

Virus infections in utero or the first years of life, early introduction of cow milk proteins, nitrosamine contents in food, cold environment, blood group incompatibility between mother and child, psychological stress and early weight gain are all risk factors for development of diabetes in childhood¹²⁻¹⁷. However, the causal relationship remains uncertain and prospective or randomized controlled studies are necessary to further explore the associations. Until today there is few such studies, but a large ongoing randomized controlled multicentre study, TRIGR, will hopefully elucidate the importance of introduction of cow milk protein during the first six month of life¹⁸. In the ABIS study (All Babies In south east Sweden)¹⁹, which started in 1997, more than 17 000 children will be followed from birth onwards to make possible further investigation of environmental factors.

Epidemiology

The incidence rate of Type 1 diabetes varies between countries worldwide from 0.1/100 000 children below the age of 15 in low incidence countries as China and Venezuela to 32 – 40/100 000 in high incidence countries as Sweden, Sardinia in Italy and Finland^{20, 21}. The incidence rate has increased dramatically over the last decades in the western countries with an almost doubling of incidence in Sweden the last 20 years¹¹. In Eastern Germany and the Baltic States the incidence has increased even more steeply after the reunification and independence, where the countries went through major economical changes^{20, 22}. It has been proposed that the rapid increase of diabetes incidence must be explained by environmental factors and an association between modern western life style and development of diabetes^{23, 24}. The Swedish Childhood Diabetes Register has noted a shift to younger age groups, in part explaining the higher incidence the last decades^{11, 23}. On the contrary, the incidence of Type 1 diabetes in the age groups 15 – 34 years, registered in the Diabetes Incidence Study in Sweden (DISS), has instead decreased during the last decades. It thus seems that the total incidence of Type 1 diabetes has not increased, but there has been a shift to younger ages²⁵.

Late complications

History

Before the discovery of insulin, Type1 diabetes mellitus was a deadly disease with a median survival of 2.5 years after diagnosis. When Banting and Best managed to isolate insulin from the beta cells in pancreas 1922 and the first patients were successful treated, one thought that the disease was cured and all problems were solved. After a period of about ten years came the first reports of severe complications from kidneys and eyes and it was realized that the diabetes disease in spite of insulin therapy could cause damage to different organs in the body²⁶. This was named late complications in contrary to acute complications as diabetic ketoacidosis and hypoglycaemia. Interestingly late complications increased in frequency after the mid - 1930s, when the insulin regimens were changed from multiple injections of rapid acting insulin every 4 to 6 hours day and night to more convenient longer acting insulin preparations given only once or sometimes twice a day²⁷. At the same time the diet recommendations were altered from very strict regimens with restriction of sugar and carbohydrate content in the food to a more free diet. The prevalence of late complications remained the same through the following decades²⁸⁻³⁰. Damage to the small and great vessels was thought to be the common pathway behind complications from different organs. Insufficient metabolic control and hyperglycaemia was early suspected to be an important risk factor for the development of complications³¹. However, everybody was not convinced about the importance of the relationship. It was not until the beginning of the 1990s that good metabolic control was generally accepted as an important risk factor. Intervention studies such as the Diabetes Control and Complication Trial (DCCT), the Oslo and the Stockholm

study had then showed a clear risk reduction of late complications with a good metabolic control³²⁻³⁴. However, some patients with a good metabolic control seemed to get late complications and even patients with a bad metabol control could escape severe complications. The last decades research therefore has focused on other possible mechanisms explaining the occurrence of late complications.

Classification

Late complications are often classified in macrovascular and microvascular lesions (Table 2), since early in the course there are structural changes in the vascular system. The microangiopathy is proposed to lead to abnormalities in the eye, kidney and nerves, but mechanisms other than damage to the vessels may be of equal importance for the development of complications. The microvascular complications are pathognomonic for diabetes mellitus, while macrovascular complications are the same as in other patients with CVD. Microvascular complications have been the main objectives for this study.

Macrovascular complications

Macrovascular complications have become a more important cause of morbidity and mortality in Type 1 diabetes according to longer life expectancy, since the prevalence increases with age as well as in the general population³⁵. It is now the leading cause of death in Type 1 diabetes³⁶⁻³⁸. In a Finnish study the cumulative incidence of cardiovascular disease (CVD) was 24% in patients with diabetic nephropathy and 7% in patients without nephropathy after 24 years of diabetes duration³⁵. A study from Pittsburgh in USA found a prevalence of CVD of 5% after corresponding diabetes duration³⁹. The prevalence of CVD in the background population has an impact of the prevalence in diabetic patients with higher prevalence in countries with higher rates of CVD³⁵.

There is a strong association between diabetic nephropathy, both microalbuminuria but especially overt nephropathy, and the occurrence of CVD, which is documented in many studies^{35, 40, 41}. The interrelationship with retinopathy is more controversial, especially after adjustment for coincident nephropathy⁴¹⁻⁴³.

The traditional risk factors for CVD as hypertension, overweight and dyslipidemia are the same in Type 1 diabetes as in the general population^{44, 45}.

Table 2 Classification of late diabetic complications

I Macrovascular complications

- a. Coronary heart disease
- b. Stroke
- c. Peripheral vascular disease

II Microvascular complications

- a. Diabetic nephropathy
 - b. Diabetic retinopathy
 - c. Diabetic neuropathy
 - Peripheral polyneuropathy (sensory and/or motor nerves)
 - Autonomic neuropathy
 - Mononeuropathy
-

Microvascular complications

Diabetic nephropathy

Definition Diabetic nephropathy is defined in clinical practice as the occurrence of proteinuria when other causes of kidney disease are excluded. It is often divided into microalbuminuria and macroalbuminuria or incipient and overt nephropathy, since these conditions have different prognostic significance. Five clinical stages of diabetic nephropathy can be recognized⁴⁶. The diagnostic criteria and concomitant structural changes are presented in Table 3.

Incipient nephropathy -microalbuminuria

Definition and diagnostic methods Microalbuminuria is often defined as AER of 20 – 200 µg/minute in at least two of three consecutive urine samples collected over a period of 6 – 12 months (Table 3). Collection of urine over 24 hours could be difficult to accomplish in clinical practice, especially in children and adolescents, thus other methods have been suggested (Table 4)⁴⁷. There seems to be a rather good correlation between the different methods in use⁴⁸. Semi-quantitative strip tests have a sensitivity of 80 – 90% and can serve as a first screening method, if confirmed by laboratory quantitative methods⁴⁹. Some authors have in adolescents used a lower cut-off value for AER of 7.5, 10 or 15 µg/minute, since the upper limit of AER in non-diabetic adolescents is 7 µg/minute⁵⁰.

Epidemiology The prevalence of microalbuminuria depends on the diagnostic criteria, which hampers comparison between different studies. With the most often used definition of AER of 20 – 200 µg/minute the prevalence varies from 10 to 20% after more than 10 years diabetes duration in different studies^{39, 51-57}, even if some authors have found prevalence rate of more than 30%^{58, 59}.

Prognosis Early studies reported that ~80 – 90% progressed to macroalbuminuria within ~10 years and that microalbuminuria was a useful predictor of subsequent overt nephropathy⁶⁰⁻⁶². Later studies have revised this opinion. About 30 – 60% reverse to normal, 20 – 40% remain microalbuminuric and only 15 – 25% progress to macroalbuminuria within 5 – 10 years⁶³⁻⁶⁸. The prognosis of microalbuminuria also differs with age at diabetes onset^{68, 69} and with diabetes duration⁶⁸, with a higher rate of progression to macroalbuminuria in adults than adolescents and a lower risk after very long diabetes duration. In adolescents four patterns of microalbuminuria have been described: normoalbuminuria, intermittent, transient and

Table 3 Stages in the development of renal changes in diabetic nephropathy (After Mogensen *et al*⁴⁶)

Stages	AER	Blood pressure	GFR	Structural changes
1 Early hyperfunction (at diabetes onset)	May be increased, but reversible	Normal	Elevated	Renal hypertrophy
2 Normoalbuminuria	Normal < 20 µg/min	Normal	Elevated	Increase of BMT
3 Incipient nephropathy	Microalbuminuria 20 – 200 µg/min	May increase	Elevated	Further increase of BMT. Arteriolar hyalinosis
4 Overt nephropathy	Macroalbuminuria > 200 µg/min	High	Decreasing	Pronounced abnormalities
5 ESRD	Macroalbuminuria > 200 µg/min	High	< 10 ml/min/ 1.73 m ²	Advanced glomerulosclerosis

AER = Albumin excretion rate
BMT = Basal membrane thickness

GFR = Glomerular filtration rate
ESRD = End stage renal disease

persistent microalbuminuria⁶⁸. Metabolic control was worse, BMT greater and GFR higher in the last groups. The prognostic value of current microalbuminuria in adolescents must be considered as unclear.

Overt diabetic nephropathy- macroalbuminuria

Definition Overt nephropathy was in previous studies often defined as persistent proteinuria, often measured as Albustix® 1+ (positive) in clinical practice. Today quantitative methods are normally used and overt nephropathy defined as AER > 200 µg/min (Table 3 and 4).

Epidemiology For patients with diabetes diagnosis during the 1930s a cumulative incidence of 40 – 50% after 25 – 30 years of diabetes duration was reported in several studies. The incidence decreased to about 30 – 40% for patients with diabetes onset in the 1940s, and then remained largely unchanged the following decades^{30, 39, 70-73}. However, Bojestig *et al* showed 1994 in the Linköping Diabetes Complications Study a dramatically declining cumulative incidence of nephropathy from 30% to 10% after 25 years of diabetes duration for patients with diabetes onset after 1966. The results were possible to achieve in an unselected population⁷⁴. The last years there have been reports of declining incidence from other centers also. Hovind *et al* in Copenhagen found a significant decreased cumulative incidence of 14 % after 20 years of diabetes duration in patients diagnosed in 1979 – 1981 and a clear declining trend to 19% in the cohort diagnosed during the years 1975 – 79, 10 years later than in the Linköping study⁷⁵. A population study from Northern Sweden completed in 1999 reported also a cumulative incidence of macroalbuminuria of 12% after an average diabetes duration of 29 years⁵¹. At a follow-up 1999 in Wales, Harvey *et al* found a cumulative prevalence of 20% after 15 – 29 years of diabetes durations⁵⁶. In the Eurodiab study in 1990 the prevalence was at the same level, 18% after 20 – 24 years disease duration⁷⁶. However, it is difficult to compare incidence data with prevalence data from cross-sectional studies, since the latter tend to give lower figures after long-term follow-up as a considerable proportion of the patients die prematurely⁴⁰.

Table 4 Methods for measurements of proteinuria

• Spot test (morning urine or random)		
Semi- quantitative test (strip test)		
Albustix®, Combur®, Redia ®		1+ corresponds to albumin /urine > 300 mg/l
Micraltest®		positive test if albumin /urine ≥ 20 mg/l
Quantitative methods		
Albumin/urine	normal range	< 30 mg/l
	microalbuminuria	30 – 300 mg/ml
	macroalbuminuria	> 300 mg/ml
Albumin/creatinine ratio	normal range: female	< 3.5 mg/mmol
	male	< 2.5 mg/mmol
• 24-hour urine collection		
• Timed overnight collection		
	normal range	< 20 µg/min
	microalbuminuria	20 – 200 µg/min
	macroalbuminuria	> 200 µg/min

Prognosis When macroalbuminuria is persistent it leads inevitable to gradually declining glomerular filtration rate (GFR) and end stage renal disease (ESRD). Until the last two decades the prognosis was bad with 50% of the patients reaching ESRD within 10 years⁷³. More recently the prognosis has been improved, probably thanks to more aggressive antihypertensive therapy^{77, 78} and better metabolic control⁷⁹. However, so far no therapy has shown to completely prevent ESRD, just retard the course.

Diabetic retinopathy

Definition and diagnostic methods There are numerous classification schemes in use⁸⁰, but a classification used in clinical practice is shown in Table 5. Background retinopathy is asymptomatic and not vision threatening, while proliferative changes and macular oedema can affect the visual acuity. Microaneurysm can be reversible⁸¹ even if a higher count is predictive of higher rates of proliferative retinopathy and macular oedema the next years⁸². Some decades ago ophthalmoscopy was the only way to examine the patients and with dilated pupils it had a rather good correlation to fundus stereoscopic photography⁸³. However, this method is more sensitive with higher reproducibility and has in many countries replaced ophthalmoscopy as a standard method for screening. Fluorescein angiography is an even more sensitive method and early retinal changes are possible to discover 4 years earlier on average than with fundus photographs⁸⁴.

Epidemiology The prevalence of background retinopathy is reported to be 50 – 60% after ~10 years of diabetes duration^{39, 54, 85-88} and nearly 100% after 20 years of diabetes duration^{39, 88-90}. Until end of the 1990s no studies had managed to show a decreasing trend. Then a study from southeast Sweden 1997 found a cumulative incidence of 32% after 10 years of diabetes duration, which is lower than earlier reported⁹¹. Bognetti *et al* in Italy also reported a lower prevalence of 23%⁹². The cumulative incidence of proliferative retinopathy is reported to be 30 – 40% after 20 years of diabetes duration^{28, 39, 70}, increasing to more than 60% after 35 – 40 years of diabetes duration^{28, 89}. There was no difference between patients with diabetes diagnosis between 1939 – 1959, despite decreasing incidence of nephropathy in the same population²⁸. In the Linköping diabetes complications study 1994 the incidence of severe retinopathy remained unchanged despite a declining incidence of nephropathy⁹³. However, Hovind *et al* found a decreased cumulative incidence of proliferative retinopathy after 20 years of diabetes duration from 31% in patients with diabetes onset 1965 – 1969 to 13% in patients with diabetes onset 1979 – 1981⁷⁵.

Prognosis Diabetic retinopathy is still the most common cause of acquired blindness in the Western world⁹⁴. The introduction of laser treatments have improved the prognosis substantially and can reduce the risk of vision loss both for proliferative retinopathy and macular oedema by 50%^{95, 96}. Even if previous studies not have shown a significant prevalence reduction of sight-threatening retinopathy, the prognosis concerning visual

Table 5 Stages of diabetic retinopathy

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- **Background retinopathy (simplex retinopathy)**
Microaneurysm. Dot and blot haemorrhages. Hard exudates.
 - **Preproliferative retinopathy**
Background retinopathy plus soft exudates, haemorrhages in all four quadrants, venous beading.
 - **Proliferative retinopathy**
Neovascularisation, besides background retinopathy.
 - **Maculopathy**
Macula oedema/macula ischemia.
-

function seems to have improved. Rossing *et al* found in Copenhagen a better preserved visual acuity after 15 years of diabetes duration in patients with diabetes onset after 1970⁸⁸.

Diabetic neuropathy

Definition and diagnostic methods The definition of diabetic neuropathy depends on which diagnostic procedures are used. It is important to distinguish if neuropathy is defined as subclinical without subjective symptoms or as clinical, which in turn can mean the presence of clinical symptom or clinical signs. Subclinical neuropathy can be diagnosed on the basis of tests, which can be performed in clinical settings, in the laboratory or with electrophysiological examinations (Table 6 and 7). There are a lot of different diagnostic procedures used for examination of neuropathy (Table 7)⁹⁷. The reproducibility is quite low and there are few studies on healthy children and adolescents which makes the use of a control group necessary in epidemiological studies^{97, 98}. Many studies have used a combination of clinical signs, tests or electrophysiological examinations to classify a patient as having neuropathy. The DCCT study required 1 of 3 clinical symptoms (physical symptoms, peripheral sensation and decreased tendon reflexes) to classify a patient as “possible clinical neuropathy”, 2 of 3 symptoms as “definite clinical neuropathy” and 2 of 3 symptoms and abnormal nerve conduction/autonomic tests for the diagnosis of “confirmed clinical neuropathy”⁹⁹. The San Antonio Conference on Diabetic Neuropathy recommended a combination of clinical symptoms, clinical examination, electrodiagnostic studies, quantitative sensory testing and autonomic function testing to fully classify diabetic neuropathy⁹⁷.

Epidemiology The prevalence of neuropathy depends on the diagnostic criterion used which varies between studies and makes comparisons difficult. The prevalence of peripheral neuropathy in young adults with inclusion of clinical criteria is ~ 20 – 30% after 10 – 20 years of diabetes duration with a wide variation between studies. The prevalence in children seems to be lower ~ 2 – 5%, but there are few studies so far. If only subclinical criteria are used, the prevalence is higher, in some studies reaching ~ 60% after just a few years of diabetes duration (Table 8). The prevalence of autonomic neuropathy also varies from just a few

Table 6 Clinical symptoms and signs of diabetic neuropathy

I Peripheral polyneuropathy

• **Clinical symptoms**

Dysaesthesia
Numbness
Pain
Muscle weakness

• **Clinical signs**

Absent tendon reflexes
Abnormal vibration (tuning fork)
Abnormal perception of pinprick

II Autonomic neuropathy

• **Clinical symptoms**

Constipation/ Diarrhoea
Vomiting
Postprandial bloating
Impotence
Postural hypotension
Hypoglycaemia unawareness

• **Clinical signs**

Table 7 Diagnostic methods regarding diabetic neuropathy

- **Peripheral polyneuropathy**
 - Quantitative sensory testing**
 - Vibration threshold (biothesiometry)-VPT
 - Tactile threshold
 - Thermal (cold/warm) threshold
 - Electrophysiological tests**
 - Nerve conduction velocity**
 - Motor nerve conduction velocity (n.medianus, n.peroneus, n.ulnaris)
 - Sensory nerve conduction velocity (n.medianus, n.ulnaris, n.suralis)
 - Action potential amplitude**
 - Compound muscle action potential amplitude (n.peroneus)
 - Sensory nerve action potential amplitude (n.suralis)

- **Autonomic neuropathy**
 - Test of heart rate control** (mainly parasympathetic)
 - HR deep breathing
 - HR E/I ratio
 - HR Valsalva ratio
 - HR at rest variation (ECG)
 - Spectral analysis of HR
 - HR lying-standing
 - Test of blood pressure control** (mainly sympathetic)
 - Postural change in systolic blood pressure
 - Change in systolic pressure sustained handgrip
 - Test of sudomotor control**
 - Temperature-induced sweating
 - Chemically induced sweating
 - Pupillometry**

HR = Heart rate E/I = Expire/inspire

percentages to more than 50% depending on diagnostic tests and study population (Table 9).

Prognosis There are few prospective follow up studies concerning the long term significance of early subclinical neuropathy. The findings seem in part to be reversible. Donague *et al* followed a group of adolescents with repeated assessments for 3 years and found very few cases of persistent abnormalities¹⁰⁰. Solders *et al* followed a group of children for 10 years after onset of diabetes. Low sensory nerve conduction and autonomic dysfunction which were common at onset, improved during the first 2 years, but deteriorated again after 10 years of diabetes duration¹⁰¹. Studies concerning the interrelationship with other forms of diabetic complications have given conflicting results. Some authors have found a strong correlation^{59, 102, 103}, while other have failed to demonstrate a connection^{104, 105}. For example, the association could be explained by an under-lying common factor, metabolic control, but Torbjörnsdotter *et al* suggested that autonomic dysregulation could be a pathogenic factor for the development of nephropathy¹⁰⁶. Adults with symptoms and signs of autonomic neuropathy have a higher mortality in CVD and sudden death¹⁰⁷.

Table 8 Prevalence of peripheral polyneuropathy

Study	Country	Year	Age Mean \pm SD (range) years	Diabetes duration Mean \pm SD (range) years	Diagnostic criteria	Prevalence %
Orchard ³⁹	USA	1989	<29 \geq 30	(20-24)	Definite clinical	33 51
DCCT ⁹⁹	USA	1989	26 \pm 7 27 \pm 7	2.6 \pm 1.4 8.6 \pm 1.7	Definite clinical	5 13
Ziegler ¹⁰⁸	Europe	1993	(11-69)	10 (0-55)	Clinical	17
Tesfaye ¹⁰⁹	Europe	1996	33 \pm 10	15	Clinical	28
Young ¹¹⁰	Scotland	1983	Teenagers (16-19) years	5 (0.5-17)	Subclinical (NC)	72
Käär ¹¹¹	Finland	1983	13 (5-19)	< 10 > 10	Subclinical (NC)	22-26 63
Chiumello ⁸⁵	Italy	1989	(15-20)	10	Subclinical (NC)	20
DCCT ⁹⁹	USA	1989	26 \pm 7 27 \pm 7	2.6 8.7	Subclinical (NC)	21 45
Donaghue ¹⁰⁰	Australia	1989	15 (13-16)	7 (4-10)	Subclinical (VPT, thermal threshold)	28
Bognetti ⁹²	Italy	1997	16 \pm 4	(11-13)	Subclinical (NC)	25
Hyllienmark ¹¹²	Sweden	1997	15 (7-20)	8 (3-17)	Subclinical (NC)	56
Solders ¹⁰¹	Sweden	1997	16 (9-21) 21 (14-26)	5 10	Subclinical (NC)	6-42 6-36
Barkai ¹¹³	Hungary	1998	14 (6-18)	6 \pm 3	Subclinical (CPT)	23
Bao ¹¹⁴	Hong Kong	1999	13 (4-21)	7	Subclinical (NC)	68
Karavanaki ¹¹⁵	England	1999	13 (4-17)	3 (0.1-13)	Subclinical (VPT)	6
Olsen ⁵⁴	Denmark	1999	21 (12-24)	13 (9-25)	Subclinical (VPT)	63

NC = Nerve conduction examinations VPT = Vibration perception threshold
CPT = Current perception threshold

Table 9 Prevalence of autonomic neuropathy

Study	Country	Year	Age Mean \pm SD (range)years	Diabetes duration Mean \pm SD (range)years	Diagnostic criteria	Prevalence %
Young ¹¹⁰	Scotland	1983	Teenagers (16-19)	5 (0.5-17)	CVT	31
DCCT ⁹⁹	USA	1989	26 \pm 7 27 \pm 7	2.6 \pm 1.4 8.6 \pm 1.7	HR test and postural BP	3 7
Donaghue ¹⁰⁰	Australia	1989	15 (13-16)	7 (4-10)	CVT	30
Ziegler ¹⁰⁸	Europe	1993	33 (11-69)	10 (0-55)	HR test	17
Solders ¹⁰¹	Sweden	1997	16 (9-21) 21 (14-26)	5 10	HR test	19-47 14-56
Karavanaki ¹¹⁵	England	1999	13 (4-17)	3 (0.1-13)	Pupillometry HR test	8 16

CVT = Cardiovascular tests HR = Heart rate BP = Blood pressure

Table 10 Possible risk factors for late diabetic complications

- Duration of diabetes
 - Metabolic control
 - Gender
 - Age at onset
 - Puberty
 - Lipid profiles
 - Systolic or diastolic blood pressure
 - BMI
 - Smoking
 - Genetic factors
-

Risk factors

Several factors have been suggested to be of importance for the development of long term complications in Type 1 diabetes (Table 10). Duration of diabetes and metabolic control are now well established risk factors, while the other associations have been more controversial with partly contradictory findings in different studies. The causal relationship between these factors and the development of complications is unclear and a better term would be risk markers. These vary partly with type of complications speaking in favour of different pathogenetic mechanisms in different organs. The importance of different risk factors also differs in patients with short or long diabetes duration¹¹⁶. Diverging results in different studies probably reflect not only the heterogeneity of the study populations, but also different adjustment for confounding factors, since there is a complex interrelationship between risk factors.

Duration of diabetes

Practically all studies demonstrate increasing prevalence of severe complications after longer diabetes duration, but the pattern differs between nephropathy, retinopathy and neuropathy.

The incidence of overt nephropathy begins to increase after 10 years of diabetes duration with an incidence peak after 15 – 17 years and then declines to a constant low level after 25 – 30 years of diabetes duration^{30, 72, 73}. The connection between microalbuminuria and diabetes duration is not as well established with different results in different studies, probably explained by different study populations and different ages at diabetes diagnosis. However, the prevalence of microalbuminuria seems to rise after 5 – 10 years of diabetes duration and then remains at a fairly constant level after 15 – 20 years of diabetes duration^{39, 53, 57, 117-119}.

On the contrary, the incidence rate of diabetic retinopathy begins to increase after 10 years of diabetes duration and after 20 years diabetes duration it is constant causing a steadily increasing prevalence during the following years of both background and proliferative retinopathy^{28, 39}.

The relationship between neuropathy and diabetes duration is more unclear, perhaps partly due to different definitions. There is undoubtedly a positive correlation between severe neuropathy with clinical symptoms and longer diabetes duration^{39, 109, 120}, but subclinical signs of neuropathy are already detected in the first years after diabetes diagnosis^{101, 121} and the prevalence in many studies then remains unchanged^{85, 100, 112}.

The prevalence of macrovascular complications also increases with duration and age as well as in the general population. These complications are unusual before 15 – 20 years of diabetes duration when diabetes is diagnosed in childhood^{35, 39}.

Metabolic control

A strong relationship between metabolic control and long term complications was found in many studies during the 1980s, when the introduction of the HbA_{1c} method made it possible to objectively measure long term glycaemic control^{31, 79, 86, 89, 90, 105, 110, 111, 120, 122-127}. The utmost importance of good metabolic control to avoid long term microvascular complications was convincingly confirmed by intervention studies as the DCCT³², the Oslo study³³ and the Stockholm study¹²⁸. In the DCCT study 1 444 patients with Type 1 diabetes for 1 – 15 years and with absence of severe diabetic complications were followed in mean 6.5 years and randomized to conventional therapy with one or two daily insulin injections or to intensive therapy with multiple insulin injections or insulin pump. The mean HbA_{1c} in the conventional therapy group was 9.1% and in the intensive therapy group 7.2%. Intensive therapy reduced the risk for progression of retinopathy 63% and laser treated retinopathy 51%. The risk of progression to all grades of albuminuria was reduced 39%, of macroalbuminuria 54% and the risk of progression to clinical neuropathy was reduced 60%³².

There has been debated whether there is a threshold for HbA_{1c} and the risk of diabetic complications. In the DCCT study it was not possible to find a threshold value of HbA_{1c} for retinopathy progression, microalbuminuria or clinical neuropathy. The relative risk reductions with 10% lower mean HbA_{1c} was the same for patients with HbA_{1c} higher and lower than 8.0 %. The recommended goal for treatment was “achieving normal glycaemia as early as possible in as many IDDM patients as is safely possible”¹²⁹, which also is the last recommendation from ADA¹³⁰. The EURODIAB Prospective Complications study group could not either confirm the existence of a glycaemic threshold for microalbuminuria¹¹⁷. On the contrary, the Berlin Retinopathy Study found a continuous exponential relationship between glycaemic control and risk for background retinopathy, with a very low risk below HbA_{1c} levels < 9.0% arguing for considering it as a threshold value in clinical practice¹³¹. Krolewski *et al* found an abruptly increased risk for microalbuminuria when HbA_{1c} was higher than 8.1%¹³².

There is a high correlation between HbA_{1c} and mean blood glucose the last 6 – 8 weeks¹³³. However, there is also a biological variation between individuals. Some individuals with the same mean blood glucose have consistently higher HbA_{1c} and others consistently lower than expected, perhaps partly genetically determined^{134, 135}. In the DCCT study patients with higher than predicted HbA_{1c} had a three times higher risk of retinopathy and six times higher risk of nephropathy compared with patients with lower than predicted HbA_{1c}. Perhaps this can be one explanation why some patients with high HbA_{1c} can escape complications and some patients with low HbA_{1c} are affected¹³⁶.

The importance of metabolic control for the development of macrovascular complications is not equally well documented and many studies have failed to find an association^{43, 45, 137}.

Gender

The effect of gender on the risk of microvascular complications is partly controversial. Many studies have shown an increased risk for overt nephropathy in males^{30, 72}, but not in all studies^{138, 139} and the results seem not to be constant over duration and age-groups³⁹. On the contrary, the prevalence of microalbuminuria seems to be higher in females^{52, 119, 140}, but this

is not confirmed by other authors^{103, 117, 138, 141}. The prevalence of retinopathy and neuropathy is most often reported as alike in both sexes^{91, 101, 103, 120, 126}, but a higher prevalence is found both in males^{89, 127} and females⁸⁷ in other studies. The conflicting results could possibly be explained by differences in metabolic control between sexes, which in turn differ between centres. Variations of age at onset, age at follow up, diabetes duration, medical care and other backgrounds factors in the community could certainly be of importance for the differences noted.

The risk for CVD in the general population is significantly higher for men. However, women with Type 1 diabetes seem to have an equal risk as men for this complication, abolishing the higher mortality rate in CVD for men and explaining higher relative mortality for women with Type 1 diabetes^{35, 38, 39, 45, 142}. The reason for this is not determined.

Age at onset and puberty

Severe complications almost never occur before puberty. For unknown reasons there seems to be a resistance to the development of late complications during the prepubertal period, even if less serious complications such as microalbuminuria, background retinopathy and subclinical neuropathy are reported^{84, 92, 143-145}. It was therefore a common conception, that the years before puberty did not contribute to the risk of long term complications¹⁴⁵⁻¹⁴⁷. However, studies the last years with longer follow up have clearly shown that the duration of diabetes before puberty is of importance, but to a lesser degree than the years after puberty^{73, 148-151}. That means that there is a prolonged time before onset of retinopathy or nephropathy when diabetes is diagnosed before puberty. Even the metabolic control during the prepubertal period seems to be of importance and Svensson *et al* showed that the metabolic control during the first five years is of independent significance for the development of complications⁵⁵. Some studies have even shown an increased risk for diabetes complications when diabetes is diagnosed during puberty compared to the prepubertal and postpubertal period^{30, 152-154}. The reason is unknown, but both a direct damaging effect of the sexual hormones and disturbed IGF1-GH-system¹⁵² on the microvascularisation in the kidney and eyes and an indirect effect via increased insulin resistance and worse metabolic control have been suggested^{119, 155}. Psychosocial factors could certainly also be of importance for the difficulties to achieve good metabolic control during the teenage period^{156, 157}.

Lipid profiles

In general the lipid profiles seem to be the same in diabetics as in nondiabetic individuals¹⁵⁸. There is a well known association between dyslipidemia and overt diabetic nephropathy. Higher levels of triglycerides, total and LDL-cholesterol and lower levels of HDL-cholesterol and other aberrations of the lipoproteins are noted. The causality between dyslipidemia and microvascular diabetic complications remains still unclear. The same pattern of dyslipidemia is seen in patients with renal failure from other causes even if it is more pronounced in Type 1 diabetes¹⁵⁹, which should speak in favour of it being secondary to the kidney disease. However, alterations of lipid profiles are found in some studies to be already in the initial phases of renal involvement in microalbuminuric patients^{117, 140}. Dyslipidemia is part of the metabolic syndrome and has been suggested to be a risk factor for the development of not only nephropathy^{103, 116, 160, 161}, but also retinopathy^{103, 116, 126, 161-163} and neuropathy^{103, 109, 120}. However, other studies have failed to demonstrate an association between dyslipidemia and development of microalbuminuria^{164, 165}, retinopathy¹⁶⁵⁻¹⁶⁷ or neuropathy¹⁶⁷, when adjusting for confounding factors.

Dyslipidemia is a well established and important risk factor for development of CVD in Type 1 as well as in the general population¹⁶⁸.

Blood pressure

The importance of systolic and diastolic blood pressure for the development of microvascular complications remains controversial. Some studies support the importance of diastolic or systolic blood pressure level for development of microvascular complications^{54, 103, 163, 169}, while other studies fail to demonstrate an association^{65, 117, 138, 170}. Different studies have used different definitions of hypertension and blood pressure values are calculated for different time periods, which perhaps partly can explain the diverging results. The cause or consequence of the associations to raised blood pressure is also difficult to interpret from cross-sectional and even from prospective studies, at least concerning nephropathy. A prospective study in initial normoalbuminuric children found higher blood pressure values first after onset of microalbuminuria¹⁷¹. On the contrary, it has been demonstrated that pathological 24-hour ambulatory blood pressure precedes the development of microalbuminuria^{106, 172}. But structural changes in the kidney are present already in the normoalbuminuric phase^{173, 174}, which could theoretically induce secondary hypertension before the impaired kidney function is possible to measure in clinical practice. It is convincingly demonstrated that aggressive blood pressure therapy can retard the progression of diabetic nephropathy, both in the microalbuminuric and the macroalbuminuric phase^{77, 175}. Intervention studies concerning the effect on the progression of retinopathy or neuropathy are lacking.

BMI

BMI is calculated as weight/height² (kilogram/meter²). Overweight has been internationally defined as BMI ≥ 25.0 kg/m² and obesity as ≥ 30.0 kg/m² for adults¹⁷⁶. BMI varies with age during childhood, but calculation of iso-BMI can make comparison between different ages possible¹⁷⁷. Calculation of iso-BMI is based on centile curves and is the corresponding BMI value if the individual is 18 years old. Central obesity is regarded as a part of the metabolic syndrome with a high correlation to cardiovascular morbidity and mortality¹⁷⁸. Other components are hypertension, dyslipidemia and insulin resistance^{179, 180}. BMI has been regarded as a satisfactory measure of obesity with correlation to insulin resistance, even if waist-to hip ratio now have been proposed to be a better risk marker¹⁸¹. Both the metabolic syndrome¹⁶⁰ and BMI have been found to have an association to diabetic nephropathy^{65, 117, 140}, retinopathy^{162, 182} and neuropathy¹⁶⁹. Other authors have failed to find an association, when adjusting for metabolic control¹⁶⁷.

Smoking

The prevalence of tobacco use has declined substantially the last decades in Sweden, but still 19% of the Swedish population are daily smokers (Official statistics, The National Board of Health and Welfare in Sweden). This is a lower proportion than in many other countries, where figures of 30 – 40% are not unusual (WHO tobacco control database). Despite efforts to encourage non-smoking habits in diabetic patients the prevalence of smokers seems to be the same as in the general population^{183, 184}. There is a well known association between smoking and CVD in non-diabetic individuals, but smoking as a risk factor in Type 1 diabetes is not as well established^{44, 185, 186}. The connection between microvascular complications and smoking are also controversial, especially concerning retinopathy. Some authors have found a clear association between progression of nephropathy and smoking and even between retinopathy, neuropathy and smoking^{34, 138, 184, 187-194}. Others have failed to demonstrate this connection^{65, 184, 195, 196}. The diverging results could in part be explained by not adjusting for

the possible confounding effect of metabolic control in some studies^{190, 197}. Psychosocial factors could speculatively affect both smoking habits and the possibility to achieve a good metabolic control^{190, 198}.

Hereditary factors

Several studies have found familial clustering of diabetic nephropathy with a 2 – 5 times higher risk for siblings and also a higher risk when the parents are affected¹⁹⁹⁻²⁰². Shared environmental factors or a genetic susceptibility could account for the relationship, with most studies supporting the last explanation. The DCCT study found familial clustering not only in diabetic nephropathy but also in retinopathy²⁰³. Studies concerning the importance of heredity for hypertension or CVD are more diverging with some studies supporting an increased risk for nephropathy²⁰⁴⁻²⁰⁸, while others have failed to find a connection between these hereditary factors and nephropathy²⁰⁹ or retinopathy²⁰⁴. The same is true for Type 2 diabetes with conflicting results in different studies. Fagerudd *et al* found a three-fold increased risk for nephropathy²¹⁰, while Roglic *et al* in the EURODIAB study found an increased risk only for albuminuria in women, but not for retinopathy, when the parents had Type 2 diabetes²⁰⁴.

C-peptide

The proinsulin molecule is produced in the beta cells in pancreas. It consists of the A- and B-chains of the insulin molecule joined by the connecting peptide, often named C-peptide. When proinsulin is released from the beta cells, the C-peptide is split from the proinsulin molecule and secreted in equimolar amounts with insulin into the portal circulation. In contrast to insulin, it is not extracted by the liver but excreted by the kidney. It has a halftime in the circulation 2 to 3 times that of insulin²¹¹. When Steiner first discovered it in 1967, it was not possible to find a biological activity of the molecule. Its function was only supposed to be facilitating the folding of the insulin molecule. It was regarded as an inert molecule, but a good marker of endogenous insulin secretion²¹².

C-peptide can be measured in serum and in urine. Different studies have used different methods which makes comparisons difficult, even if there is a rather good correlation between urinary C-peptide, fasting and stimulated serum C-peptide values²¹³⁻²¹⁶. C-peptide secretion can be stimulated by glucagon injection or more physiologically by a mixed meal. Both standardized breakfast and a liquid meal (Sustacal®) with fixed composition of fat, proteins and carbohydrates have been used in different studies. The ADA (American Diabetes Association) Workshop 2001 recommended measurement of C-peptide as the primary outcome in clinical trials to preserve beta cell function and that measurement of both fasting C-peptide and stimulated C-peptide has to be performed²¹⁷.

With help of C-peptide analysis, many studies in the 1970s and 1980s examined the natural history of insulin secretion after newly diagnosed diabetes. However, most of the C-peptide studies are cross-sectional or have followed the patients prospectively for a rather short period, seldom longer than one or two years. An astonishingly great part of the patients was found to preserve C-peptide secretion for many years. At diagnosis still 70 – 100% of the patients had measurable C-peptide in blood or urine²¹⁸⁻²²¹. The highest values of C-peptide were often found after 3 months of diabetes duration and then gradually declined²²²⁻²²⁴. This period coincided with the well-known clinical phenomenon of “honeymoon period” with a low insulin requirement and good metabolic control. After 2–3 years of diabetes duration still 30 – 40% have measurable C-peptide levels²²⁴⁻²²⁷, but the prevalence is then rapidly declining to 10 – 15% after 5 – 10 years^{224, 227, 228}. However, it is difficult to compare results in different studies because of a great variation of C-peptide secretion depending on the study population.

Practically all studies have shown that adults and elderly children have higher values of C-peptide during a longer period^{6, 220, 222-224, 227-231}. The connection with gender remains controversial, some studies showing a longer persistence of C-peptide secretion in males²²⁹ than in females, while other studies have found the opposite^{219, 224}. From a theoretical point of view, one could expect an association between diabetic ketoacidosis (DKA) at diagnosis and lower C-peptide values also described by some authors^{220, 224, 232}, while others have failed to find a connection^{219, 222, 223, 233}. The same is valid concerning DKA at diagnosis and length of persistent C-peptide secretion^{219, 220, 222-224, 232, 233}.

Some small early studies in the 1970s and 1980s showed a longer remission period with more intensive insulin regimens at diagnosis^{231, 234, 235}. However, other studies have not confirmed this influence on C-peptide secretion in a longer term by different mode of insulin delivery at onset of diabetes²³⁶⁻²⁴⁰.

Several studies have demonstrated an association between higher values of fasting or stimulated C-peptide and better metabolic control measured as lower HbA_{1c}. In the same studies there is also a connection between persistent C-peptide secretion and lower insulin doses^{218, 226, 241, 242}. However, Sochett *et al* could not confirm these results²¹⁹. From cross-sectional studies the cause and consequence relationship remains unclear. In the DCCT study, patients with persistent C-peptide secretion at entry had lower HbA_{1c}. The intensively treated group with HbA_{1c} 7.2% had a higher probability of maintaining C-peptide secretion (57% risk reduction) compared with the conventionally treated group with HbA_{1c} 9.1%. This speaks in favour of that intensive therapy helps to sustain endogenous insulin secretion, but the study cannot entirely exclude the possibility that persistent C-peptide secretion can contribute to a better metabolic control²⁴².

In recent years there has been an increasing interest in the research field of C-peptide secretion and the possibility that persistent C-peptide secretion could prevent long term complications. In rat and in vitro experiments there have been indications of the presence of a C-peptide receptor on the cell surface²⁴³. C-peptide has been found to stimulate Na-K-ATPase activity²⁴⁴ and subsequent stimulation of eNOS (endothelial nitric oxide synthase) activity²⁴⁵. In human experiments infusion of C-peptide during 2 hours in physiological amounts in 11 patients with Type 1 diabetes and glomerular hyperfiltration resulted in 7% decrease of GFR and 3% increase of renal plasma flow compared to controls. The glucose transport in skeletal muscle increased and the whole body glucose utilization rose 25%²⁴⁶. In another experiment infusion of C-peptide during 3 hours in 12 patients with autonomic nerve dysfunction improved heart rate variability 63%²⁴⁷. It has also been possible to demonstrate increase of nutritive skin microvascular blood flow²⁴⁸. However, these experiments are short time effects of C-peptide administration. In a randomized controlled trial 21 patients with Type 1 diabetes and microalbuminuria received subcutaneous injection of C-peptide together with insulin during 3 months. AER decreased 40%, from 58 µg/min to 34 µg/min, despite unchanged HbA_{1c}. In a subgroup of patients with signs of autonomic nerve dysfunction, respiratory heart variability as well as temperature threshold improved²⁴⁹. In another study 26 diabetic patients received C-peptide injections for 3 months and showed improved sensory nerve conduction and lower VPT, but unchanged motor nerve conduction velocity and thermal perception²⁵⁰. These rats, in vitro and human studies show that C-peptide has a biological effect. However, these experiments are short term studies with few patients and with substitution of C-peptide in physiological amounts. They cannot answer the question of the clinical importance of residual C-peptide secretion at diabetes diagnosis. Only long term randomized controlled

trials with simultaneous injections of insulin and C-peptide can answer the question if C-peptide *per se* has a beneficial effect on the development of long term complications.

The importance of persistent C-peptide secretion on long term complications could hypothetically depend on a direct effect of C-peptide on endothelial cells and nerve cells or an indirect effect via endogenous insulin secretion. The latter could possibly promote a better metabolic control or have an influence via the IGF-1-system in the liver, since portal insulin delivery seems to be needed to normalize the IGF system²⁵¹. There are some studies which indicate that the IGF system influences the development of long term complications²⁵². There are just a few clinical studies demonstrating a beneficial effect of persistent C-peptide secretion on the prevalence of long term complications. Ludvigsson *et al* found a positive correlation between fasting C-peptide and sensory nerve conduction velocity and vibratory sensibility in children, but it was before the possibility to measure HbA_{1c}²⁵³. On the contrary, Hyllienmark *et al* found a weak negative correlation between nerve conduction velocity in the median nerve and duration of C-peptide secretion in children¹¹². Kernell *et al* found a negative correlation between abnormal vitreous body leakage and persistent C-peptide even when adjusting for HbA_{1c}²⁵⁴. A number of population studies²⁵⁵⁻²⁶¹ have not confirmed a lower prevalence of diabetic retinopathy, nephropathy or neuropathy in patients with C-peptide secretion with the exception of a few studies which did not adjust for metabolic control^{256, 262}.

In the DCCT study the patients in the intensive treatment group with C-peptide secretion ≥ 0.2 nmol/l at entry had a 50% risk reduction for retinopathy progression and 27% risk reduction for nephropathy progression during the following 6 years. When adjusting for HbA_{1c} the differences were no longer statistically significant. The DCCT study concluded that intensive therapy helps to sustain insulin secretion which is associated with better metabolic control and consequently a lower risk for complications. However, the glycaemic control is probably a more important factor than the direct effect of the persistent C-peptide secretion *per se*. However, patients with persistent C-peptide secretion had a 62% risk reduction for hypoglycaemia, even after adjustment for HbA_{1c} which could be of great clinical benefit for the patients²⁴².

Mortality

The mortality rate is increased in Type 1 diabetes and varies worldwide with higher standardized mortality ratios (SMR) in Eastern Europe and Japan, countries with a low incidence of Type 1 diabetes^{263, 264}. There seems to be a positive correlation between the general mortality rate in different countries and excessive mortality due to diabetes. This is probably explained by lower socioeconomic conditions and lower standard of medical care, but perhaps in part also by lower incidence of diabetes in these countries. When diabetes is more infrequent, there could be a higher risk of incorrect therapy for acute complications.

The mortality rate has declined over the last decades, both during the first years after diagnosis and after longer diabetes duration^{78, 142, 175, 265-268}. Still, the SMR is doubled in young people with diabetes and in many studies is 3 to 4 times higher than in the general population after longer diabetes duration^{37, 38, 269-273}. The most frequent causes of death in patients younger than 30 years are acute complications such as DKA and hypoglycaemia^{37, 268, 270, 274, 275}. Psychosocial and socioeconomic risk factors seem to be of importance for the mortality in these younger age groups, but are of lesser significance for mortality due to chronic complications in the elderly age groups²⁷⁶. The strongest risk factor for premature death after longer diabetes duration is persistent proteinuria^{36, 40, 72, 277}. The excessive mortality in Type 1

diabetes is mostly found among patients with macroproteinuria. Even microalbuminuria is a predictor of increased mortality, if not in the same degree as macroalbuminuria^{36, 277, 278}. Beside diabetic nephropathy, smoking and hypertension are well recognized risk factors for premature death as well as in the general population^{36, 277}. The dominant causes of mortality in patients older than 30 years are CVD or ESRD³⁷. Some decades ago the prognosis in patients with overt nephropathy was very bad with a median survival time of 5 – 7 years after onset of persistent proteinuria⁷². Even if uraemia was the main cause of death, cardiovascular deaths were also more frequent in these patients⁴⁰. The prognosis has steadily improved and Astrup *et al* reported in 2005 that the median survival time after onset of overt nephropathy during the last decades has increased to 22 years. The reason is probably a more aggressive treatment of hypertension^{175, 279, 280}. About half of the deaths were caused by ESRD and 40% by CVD.

Ethical considerations

When the study concerning C-peptide secretion (Study III) started with collection of data in 1975, it was performed according to the ethic codes of the Declaration of Helsinki, which first version was published 1964. The patients and their parents were verbal informed about the study and gave their consent to participate and the study was approved by The Research Ethics Committee of the Faculty of Health Sciences, Linköping University. During the last decade the participants were informed both in writing and verbally and gave a written consent to participate. The blood samples at diabetes diagnosis were taken in clinical routine and participation in the study meant no extra discomfort for the children. The blood samples for C-peptide determination were mostly taken together with clinically motivated blood samples and meant no extra venepuncture. However, the extra time of repeated blood samples for the determination of stimulated C-peptide tests were inconvenient for preschool children, and was why they were often excluded from this part of the study.

Most of the data concerning long term complications (study I, II and IV) were collected in the clinical routines and meant no extra blood samples or investigations for the patients. In study II the electrophysiological examinations could cause a light discomfort, but no pain. Since it is difficult to inform smaller children about this phenomenon, only children older than 7 years were asked to participate in this study. The inquiry to participate in a study concerning complications could possibly create alarm about the future, but on the contrary, the patients were already well informed about the risk of long term complications and the consciousness of participation in a study could help to motivate to take better care of the diabetes therapy. The patients, and for children their parents, were informed in writing about the studies and gave a written consent to participate and the Research Ethics Committee of the Faculty of Health Sciences, Linköping University approved the studies.

Aims of the study

A previous study from the Linköping area has shown a remarkable decreased incidence of diabetic nephropathy, but unchanged incidence of severe retinopathy, after 20 years of diabetes duration during the last decades. The insulin therapy during this period changed from conventional therapy with one or two insulin injections daily to intensive therapy with multiple insulin injections and also more intensive insulin regimens at diabetes onset. Despite intensive insulin treatment from onset since the midst of 1980s, in a previous study we found a high prevalence of subclinical neuropathy after the first ten years after diabetes diagnosis. The incidence of Type 1 diabetes in childhood has increased rapidly the last two decades. The reason must be environmental factors, which also might lead to different disease process and manifestation.

The aims of the studies were to

- ❖ Analyse if the decreased incidence of diabetic nephropathy persisted after 10 more years of follow up. Have we prevented or just postponed diabetic nephropathy?
- ❖ Analyse the incidence of severe retinopathy. It is still unchanged after longer follow up?
- ❖ Analyse the importance of possible risk factors for development of microvascular complications, which could explain the improved prognosis in our population.
- ❖ Analyse whether intensive treatment from diabetes diagnosis is more effective in preventing other microvascular complications than neuropathy.
- ❖ Investigate the relationship between subclinical neuropathy and diabetic nephropathy and retinopathy.
- ❖ Examine C-peptide secretion the first years after newly diagnosed diabetes and the relationship to clinical characteristic at onset, initial therapy, length of partial remission period and metabolic control.
- ❖ Investigate if there was any secular trend of C-peptide secretion and prevalence of remission period.
- ❖ Analyse if longer persistence of C-peptide secretion is of importance for prevention of diabetic nephropathy or retinopathy.

Methods

Study population

The whole study population consists of all 478 patients with Type 1 diabetes diagnosed before the age of 15 years during 1961 – 2000 living in the catchment area of the Paediatric Clinic, University Hospital, Linköping, Sweden. They represent a total unselected population as in Sweden all children with diabetes are treated at a paediatric clinic. For the analysis the patients were divided into eight five-year cohorts according to the year of diagnosis of diabetes. The groups were similar in mean age at onset, proportion diagnosed before puberty (defined as < 11 years for girls and < 12 years for boys) and sex distribution (Table 11). The incidence of Type 1 diabetes doubled during the last 25 years (Fig 1).

During the 1960s the standard regimen was a single dose of long acting insulin in the morning. The number of daily insulin injections increased gradually during the 1970s and 1980s to 4 or 5 with a combination of short acting and long acting insulin. In the 1990s several patients used insulin pumps. Multiple insulin injections therapy already from start was introduced in the late 1980s, even for young children (Fig 2a). Since the late 1980s a majority of patients was treated initially with intravenous insulin for at least 2 – 3 days irrespective of the severity of clinical presentation. The standard therapy before was repeated doses of subcutaneous insulin (Fig. 2b). In the 1970s a diabetes team with doctors, nurses, dietician, psychologist and social worker was introduced at the paediatric clinic. The patients were strongly recommended regular self-monitoring of urine glucose and after 1980 self-monitoring of blood glucose. After 1980 regular measurement of HbA_{1c} gave additional possibility for the patient to optimise metabolic control, which was one of the main goals of the therapy. The same routines were followed when the patients at the age of 18 to 20 years were transferred to the Department of Internal Medicine. One fifth of the patients had moved one to several years after diabetes diagnosis. The insulin regimens and main goal of treatment have during the last decades been about the same in other hospitals in Sweden, even if there were minor differences in management of diabetes.

All 269 patients with diabetes onset 1961 – 1985 participated in **study I and IV**. Three patients moved abroad within 5 years after diabetes diagnosis. In total 25 patients have died,

Table 11 Clinical characteristics of patients with Type 1 diabetes diagnosed in childhood

Period of onset	n	Age at onset	Male	Onset before puberty	Patients followed until 1997	Patients followed until 1997	Long term HbA _{1c}	Participants in study number
		Years Mean (SD)			%	retinopathy		
1961-1965	56	8.5 (3.5)	41	71	52 (92.9)	55 (98.2)	7.5 (0.9)	I + IV
1966-1970	50	8.0 (3.9)	48	76	43 (86.0)	48 (96.0)	7.4 (0.8)	I + IV
1971-1975	55	8.7 (4.0)	53	60	52 (94.5)	52 (94.5)	7.4 (0.9)	I + IV
1976-1980	51	8.7 (3.8)	39	69	47 (92.2)	49 (96.1)	7.3 (1.1)	I + IV, II, III
1981-1985	57	8.9 (3.8)	56	63	50 (87.7)	51 (89.5)	7.1 (0.9)	I + IV, II, III
1986-1990	53	8.9 (4.2)	62	70	–	–	7.0 (1.0)	II, III
1991-1995	66	8.5 (3.8)	59	71	–	–	6.8 (0.8)	III
1996-2000	90	8.5 (3.8)	51	74	–	–	6.4 (0.7)	III
All periods	478	8.6 (3.8)	52	70			7.0 (1.0)	

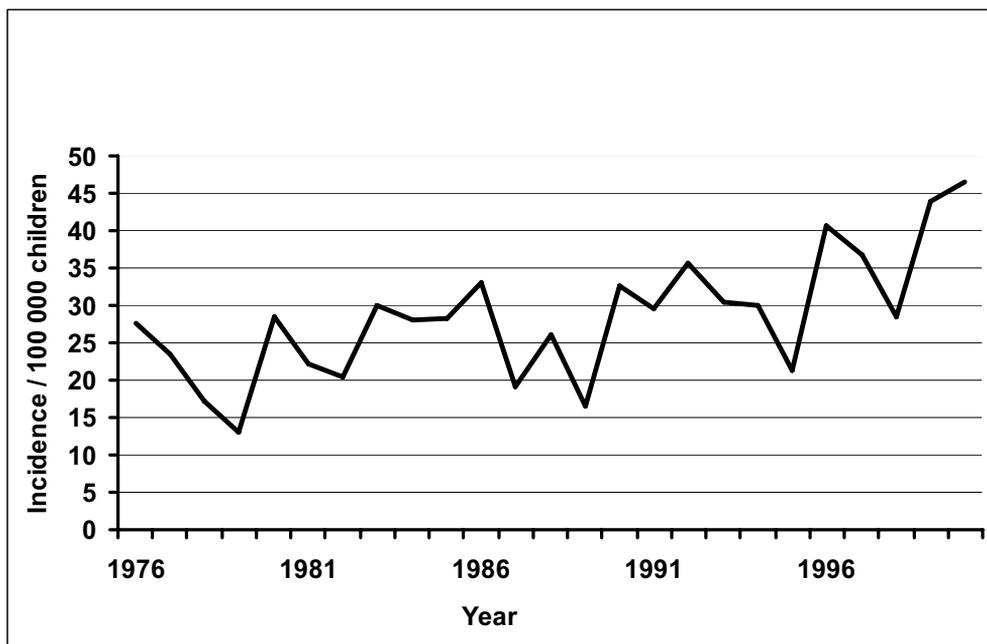


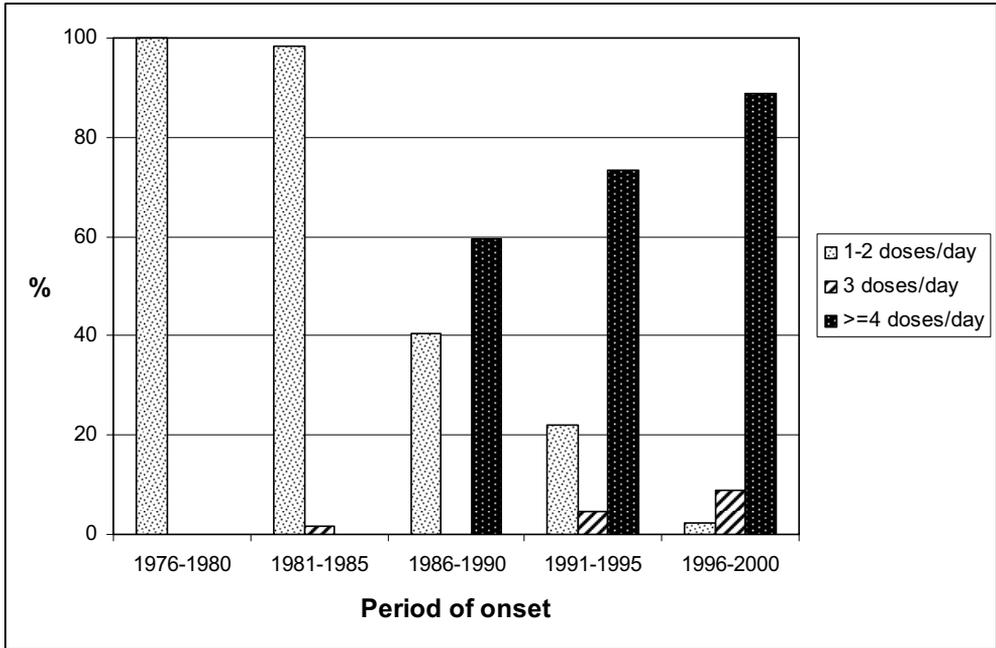
Fig. 1 Incidence of Type 1 diabetes before the age of 15 years in the Linköping region 1976 – 2000

twelve of them before developing retinopathy and seven before developing nephropathy. The population was followed until the end of the 1990s. The majority of the patients, 214 (80%), remained in the catchment area and information was collected from their medical records. For the other patients, who had left the area, information was collected by a questionnaire through their physicians. Of the total population 244 (90.7%) were monitored for retinopathy and 255 (94.8%) for nephropathy until at least 1997. The remaining patients were followed until their most recent clinical visit.

The population in **study II** consisted of all 82 patients with Type 1 diabetes more than 7 years old, attending the Paediatric Clinic in 1993, and who had diabetes duration more than 3 years. Two patients were excluded from the study, one with morbus Down and one with severe scleroderma. Five patients have moved to Linköping from other regions and thus did not belong to the other study population. The mean (SD) age at onset was 7.0 (3.6) years, range 1.0 – 16.1 years. Seventy-three patients (91 %) were diagnosed before puberty. Forty-seven (59 %) were male. All patients were treated with intensive insulin regimens from onset of diabetes. During the follow up period 12 (15 %) patients were treated with an insulin pump in median 5.1 years (range 0.3 – 8.3 years). The pump therapy began in median 9.4 years (range 2.9 – 14.2 years) after diabetes diagnosis. All patients were either in puberty or beyond when examined for eye and kidney complications.

All 316 patients with diabetes onset 1976 – 2000 participated in **study III**. The patients were followed in the study as long as they were seen at the outpatient paediatric clinic. They left the clinic at the age of 18 – 20 years, when they were transferred to the Department of Internal Medicine or previously if they moved out of the region.

a)



b)

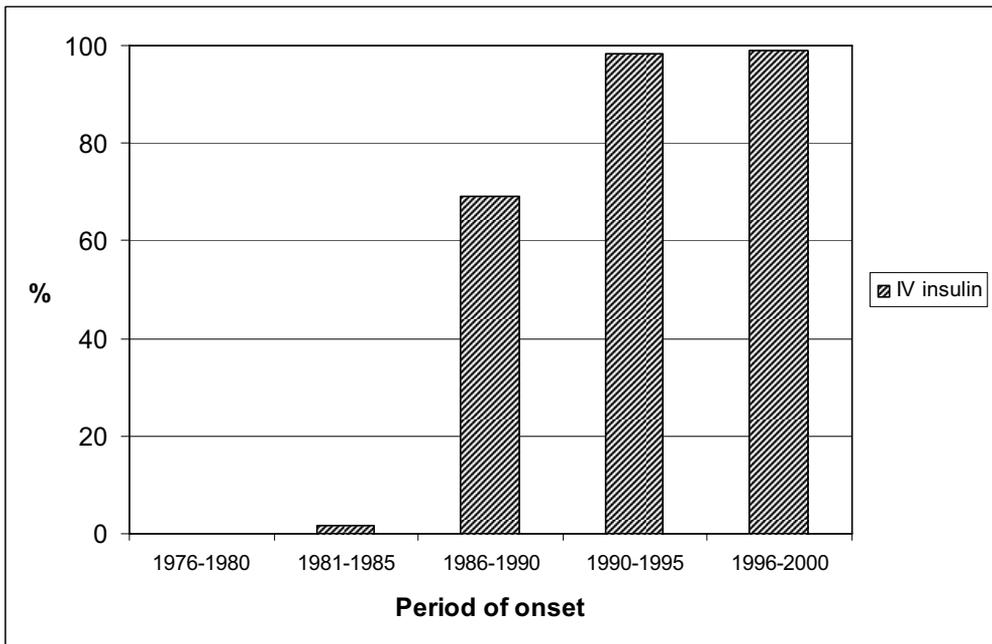


Fig. 2 Initial insulin therapy in patients with Type 1 diabetes diagnosed in childhood during 1976 – 2000 a) Percentage of patients with different numbers of insulin doses per day the first 2 years after diabetes diagnosis b) Percentage of patients with intravenous insulin therapy at diagnosis

Design

The study design is an observational study starting in 1961. Data concerning severe retinopathy and overt nephropathy are registered prospectively as part of a clinical screening programme. Blood pressure, weight, insulin doses and, since 1980, HbA_{1c} are registered at the clinical visits 3 – 4 times per year. Since 1976 C-peptide are analysed at least yearly if the patient still visits the paediatric clinic.

In **study I and IV** information about date of occurrence of severe retinopathy and overt nephropathy, CVD, long term blood pressure and long term metabolic control was collected from the patients' records in the catchment area or through a questionnaire to their physicians if they have moved. Information about all grades of retinal changes and microalbuminuria was registered 1990 and at the last clinic visit in the end of the 1990s. Cross-sectional data for lipoproteins and BMI was also collected from the records. Of the 269 patients in the total study population 183 (68%) answered a questionnaire in the end of 1990s and reported smoking habits and heredity for diabetes and CVD.

In **study II**, 79 of 80 patients participated in at least one of two electrophysiological examinations in 1993/94 or 1997/98 (Fig 3). Cross-sectional data about all grades of diabetic retinopathy and nephropathy at the last clinic visit in the end of the 1990s were collected for all patients.

Clinical and laboratory characteristics at diagnosis were collected retrospectively from medical records in **study III**. HbA_{1c}, insulin doses and weight were registered at the regular clinic visits at least three times a year.

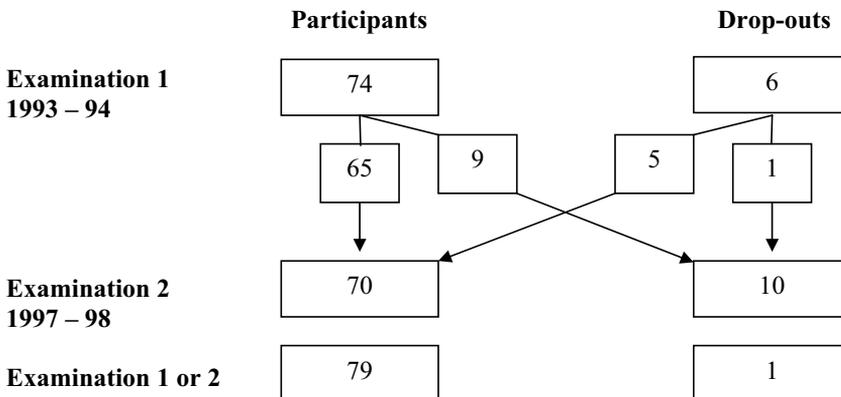


Fig. 3 Number of patients participating in the neurophysiological examinations the years 1993 –1994 and 1997 – 1998 respectively, in a population of young patients with intensively treated Type 1 diabetes

Definitions and laboratory methods

Retinopathy

The screening was performed up to the 1970s with ophthalmoscopy and later on with colour fundus photography. Three photos were taken nasally, on the macula and on the optic nerve of each eye with a photo angle of 45°. After 1995 the pictures were digitalized with 2 photos of each eye (macula and optic nerve). Additional 3 photos were taken if there was any abnormal finding. All photos were examined by ophthalmologists and in a standard protocol summarized into four classes on the basis of the worse eye, namely into normal, background retinopathy, preproliferative retinopathy and proliferative retinopathy. The presence of maculopathy was noted. The patients who had left the catchment area were examined by their local hospital. The same grading system was used, but the classification was done locally. The information was collected from the patients' medical records or their physicians.

The onset of severe retinopathy was defined as the date of the first laser treatment. The indications were proliferative retinopathy or macular oedema.

The terms diabetic retinopathy or “any retinopathy” mean the presence of all grades of retinal changes.

Nephropathy

All patients were tested for proteinuria at their regular clinic visits with a semi-quantitative test strip (Albustix®, Combur®, Redia® or equivalent strips). During the last decades the patients, at least once every year, had a morning sample of urine analysed by a quantitative immunoturbidometric method at the local hospital laboratory, either as a timed over-night analysis or as a spot test. For patients moving away to other parts of the country, analysis was made at the local hospitals with similar quantitative methods, but in a few cases with a semi-quantitative method (Micral® test).

Overt diabetic nephropathy or macroalbuminuria (in study I named just diabetic nephropathy) was defined as persistent proteinuria, that is at least 1 + test result (which corresponds to an albumin concentration in the urine of > 300 mg/l) or with quantitative method AER > 200 µg/min or albumin concentration > 300 mg/l. The onset was defined as the first year during which proteinuria became persistent.

Microalbuminuria was defined as AER 20 – 200 µg/minute or an albumin concentration of 30 – 300 mg/l in the last available urine sample.

The term “any nephropathy” means the presence of macroalbuminuria or microalbuminuria.

Neuropathy

Electrophysiological examination was performed with a standard technique using surface electrodes and the results were compared with healthy control subjects^{11,12}. Motor nerve conduction velocity and compound muscle action potential amplitude were measured in the peroneal nerves bilaterally. Sensory nerve conduction velocity and sensory nerve action potential amplitude were measured in the sural nerves bilaterally. The legs of the patients were warmed with heat pads prior to the measurements and the skin temperature held constant at 33 – 34 °C during the investigations. Individual values were considered abnormal when they fell outside the interval ± 2 SD around the mean in the control group.

Table 12 Methods for analysing HbA₁/HbA_{1c} by the hospital laboratory in Linköping during different periods and conversions formula

Period	Method	Formula
– 14 April 1982	HbA ₁ , incl labile fraction (Ion exchange chromatography, Quicksep, Isolab)	HbA_{1c} (ref) = 0,687 * HbA ₁ + 0,3322
15 April 1982 – 24 October 1984	HbA ₁ , stabile fraction (Ion exchange chromatography, Quicksep, Isolab)	HbA_{1c} (ref) = 0,687 * HbA ₁ + 0,3322
25 October 1984 – 27 April 1986	HbA ₁ , stabile fraction (HPLC, Auto A1c HA 8810, Daichi)	HbA_{1c} (ref) = 0,7554 * HbA ₁ + 0,03
28 April 1986 – 30 November 1996	HbA _{1c} (HPLC, Mono-S, Auto A1c HA 8810, Daichi)	HbA_{1c} (ref) = 0,982 * HbA _{1c} + 0,03
1 December 1996 – 30 April 2002	HbA _{1c} (Immunological, Hitachi 917)	HbA_{1c} (ref)
1 May 2002 – 14 September 2004	HbA _{1c} (Immunological, Advia 1650)	HbA_{1c} (ref)

HbA₁/HbA_{1c} = actual laboratory value

HbA_{1c} (ref) = reference value calibrated to the Swedish national standard Mono-S, normal range 3.6–5.0%

Diabetic neuropathy or nerve dysfunction was defined as abnormality in two or more nerves (of totally 4 nerves), as none of the controls had abnormal nerve conduction in more than one nerve.

Metabolic control

HbA_{1c} was measured within one week of diagnosis and then regularly at the clinical visits at least 3 – 4 times per year. All blood samples were initially analysed by the central hospital laboratory. When the method was introduced in 1980 HbA₁ was analysed with an ion exchange chromatography with a minicolumn. From 1984 a high performance liquid chromatography (HPLC) method was used, first giving HbA₁ values and from 1986 HbA_{1c}. After 1996 the method was changed to an immunological method, which was calibrated against the Swedish national standard Mono-S and continuously controlled against the Swedish EQUALIS reference method (External Quality Assurance in Laboratory medicine in Sweden). The normal range is 3.6 – 5.0%. The values can be transformed to the corresponding DCCT values by adding 1.1%²⁸¹. From 1993 most of the blood samples on the paediatric ward were analyzed by a diagnostic chemistry analyser DCA 2000, first calibrated against the hospital laboratory and since 1997, recalibrated against the Mono-S method. From the analysing laboratory there were calculated inter-method calibrations and conversion factors every time when the methods were changed (Table 12 and 13). For many of the patients, who moved it has been possible to obtain their HbA_{1c} values by their physicians and conversions factors to the EQUALIS reference method by the local laboratory. However, 94% of the HbA_{1c} values are from laboratories in the catchment area.

Table 13 Methods for analysing HbA_{1c} on the Paediatric clinic in Linköping during different periods and conversions formula

Period	Method	Formula
1 January 1993 – 31 August 1997	DCA 2000	HbA_{1c} (ref) = 0,982 * HbA _{1c} + 0,03
1 September 1997 –	DCA 2000	HbA_{1c} (ref)

HbA_{1c} = actual laboratory value

HbA_{1c} (ref) = reference value calibrated to the Swedish national standard Mono-S normal range 3.6–5.0 %

In **study II** the values were converted to the reference value for the period 1986 – 1996 with a normal range of 3.5 – 5.5%. As a measure of long term glycaemic control for each patient, the mean for each year and then the mean for the whole period until the latest nerve examination was calculated.

As a measure of long term glycaemic control for each patient in **study III and IV**, mean HbA_{1c} was calculated and weighted for the time between the measurements²⁸². In **study III** mean HbA_{1c} during 1, 3 and 5 years was calculated in the same way. In **study IV** all HbA_{1c} values until the year of onset of severe retinopathy and overt nephropathy respectively, were included in the calculations. HbA_{1c} values were not available for 17 (47%) of the 36 patients with overt nephropathy and for 17 (25%) of the 69 patients with severe retinopathy because the complications occurred before the introduction of the HbA_{1c} measurements.

Cardiovascular risk factors

Blood pressure Systolic and diastolic blood pressure was measured regularly at the clinical visits in a sitting position after a few minutes rest. Long term blood pressure was calculated as a mean of all measurements until the year of onset of severe retinopathy and overt nephropathy respectively, and weighted for different time intervals between the measurements²⁸². Blood pressure before 18 years of age was excluded because of difficulties to compare values in childhood with adult values.

Lipid profiles Lipoproteins (total cholesterol and triglycerides) were analysed at the local hospital laboratories with an enzymatic method. The reference value was < 5 mmol/l for cholesterol and < 1.7 mmol/l for triglycerides. It was not analysed regularly until the late 1990s.

BMI Body mass index (BMI) was calculated as weight (kilogram)/length (meter)² and recorded at the last follow-up 1998/99.

CVD History of cardiovascular disease (stroke, myocardial infarction, cardiac failure or symptoms of arteriosclerosis) was collected from the patients' medical records or their physicians.

Smoking History of smoking was defined as former or current smoking more than one cigarette per day as reported in the questionnaire in the end of 1990s.

Heredity Type 2 diabetes, hypertension and cardiovascular disease in the family were reported in the questionnaire.

C-peptide secretion

After 1976 serum-C-peptide was analysed at diabetes onset before the first insulin injection. Fasting serum-C-peptide before the morning insulin injection was then analysed at least yearly until the patients left the Paediatric clinic or C-peptide was not detected for 3 consecutive years. After diabetes duration of 3 months, 9 months, 1.5 years, 2.5 years and 4 years C-peptide was also analysed after stimulation with a mixed meal (standardised breakfast) without insulin injection. Blood samples were taken before eating and then at intervals of 30 minutes over a period of 120 minutes. Fasting and maximum value were registered. Because of low age or difficulties with venepuncture the participant rate varied between 81 – 93% on the different occasions. The presence of detectable C-peptide, C-peptide > 0.1 nmol/ or > 0.2 nmol/l after 1, 3 and 5 years of diabetes duration was stated. Data was not available for 5 – 10% of the patients at different times, but with the same distribution between cohorts. The serum was stored at – 20°C until analysis. C-peptide was determined until 2001 by a radioimmunoassay method²⁸³. Detection limit was 0.03 nmol/l. The fasting reference range in non-diabetic children was 0.18 – 0.63 nmol/l. After 2001 a fluoroimmunometric assay was used (AutoDELFLIA® C-peptide) with fasting reference range

0.12 – 1.2 nmol/l and the same detection limit. For technical reasons the samples collected after 2001 were stored for a longer time before analysis.

Partial remission

Partial remission period was defined as the time period with insulin requirement < 0.5 U/kg /day under good metabolic control (HbA_{1c} < 7.5%).

Diabetic ketoacidosis

Diabetic ketoacidosis (DKA) was defined as $\text{pH} \leq 7.30$ and/or base deficit ≤ -10.0 . The blood sample was analysed at the hospital laboratory with conventional methods.

Statistical analysis

Student's unpaired t-test (two-tailed) and one-way ANOVA, with Bonferroni adjusted pairwise comparisons, were used for comparisons of means between groups. Mann-Whitney U-test and Kruskal-Wallis test were used for skewed data. Chi-square or Fisher's exact test was used for comparisons of proportions.

The cumulative proportion of severe retinopathy and overt nephropathy was calculated for one-year intervals with a life table method. Patients who have not developed any complications contributed to the follow up until the last clinic visit or to the year of death. For analysing the importance of year of diabetes onset the population was divided into five-year cohorts according to the year of diabetes diagnosis. For analysing the importance of metabolic control the patients were divided into four groups according to long term HbA_{1c} . Differences between groups were tested using the Wilcoxon (Gehan) log-rank statistic test.

Logistic regression models were used in study III for analyzing the relationship between prevalence of C-peptide secretion and possible influencing factors. In study IV it was used for analyzing the relationship between any retinopathy and any nephropathy and different risk factors adjusting for the possible confounding effect of HbA_{1c} and diabetes duration. Odds ratios (OR) with 95% CI were estimated. Cox proportional hazard analysis was used to analyse the influence of the different possible risk factors for the occurrence of severe retinopathy and overt nephropathy.

Relative risk (RR) was calculated in study I for comparing prevalence of non-proliferative retinopathy and microalbuminuria in 1990 and at the end of the 1990s. In study II it was used for comparing the prevalence of retinopathy or nephropathy in patients with and without neuropathy.

The calculations were performed using the SPSS Statistical Package for Social Science ver 10.0 – 12.0 and Epiinfo™ 2002. $p < 0.05$ was considered statistically significant.

Results

Incidence of diabetic retinopathy and nephropathy

The cumulative proportion of severe laser treated retinopathy showed a significant declining trend during the last decades. The decrease was significant between the oldest cohort with diabetes onset 1961 – 1965 and the cohorts with diabetes onset 1971 – 1975 and 1976 – 1980. After 25 years of diabetes duration it was 47%, 28% and 24% in the three oldest cohorts respectively. After 30 years of diabetes duration it had increased to 53% and 44%, respectively (Fig. 4a).

The cumulative proportion of overt nephropathy also declined with a significant decrease between the oldest cohorts and all the following cohorts. After 25 years of diabetes duration it was 30%, 8% and 13% in the three oldest cohorts respectively. After 30 years of diabetes duration the cumulative proportion remained largely unchanged at 32% and 11% in the cohorts 1961 – 1965 and 1966 – 1970 respectively (Fig. 4b).

Table 14 Possible risk factors in patients with Type 1 diabetes diagnosed in childhood with and without retinopathy

Risk factor	No retinopathy <i>n</i> = 64	Background retinopathy <i>n</i> = 131	Severe laser treated retinopathy <i>n</i> = 69	<i>p</i> value
Diabetes duration (years)	19.7 (6.3)	24.3 (6.9)	31.7 (6.3)	< 0.001*
Long term HbA _{1c} (%)	6.7 (0.8) <i>n</i> = 62	7.4 (0.8) <i>n</i> = 130	7.9 (1.0) <i>n</i> = 52	< 0.001*
Systolic blood pressure (mm Hg)	120.4 (8.7) <i>n</i> = 60	123.8 (7.8) <i>n</i> = 130	128.5 (13.4) <i>n</i> = 54	< 0.001 [†]
Diastolic blood pressure (mm Hg)	74.6 (5.9) <i>n</i> = 60	76.7 (4.4) <i>n</i> = 130	80.8 (7.3) <i>n</i> = 54	< 0.001 [†]
History of smoking (%)	36.4 <i>n</i> = 44	40.8 <i>n</i> = 98	68.3 <i>n</i> = 41	0.004
BMI (kg/m ²)	24.6 (3.7) <i>n</i> = 59	25.9 (3.5) <i>n</i> = 128	25.6 (3.5) <i>n</i> = 54	0.06
Total cholesterol (mmol/l)	4.5 (0.8) <i>n</i> = 44	5.0 (1.1) <i>n</i> = 114	5.1 (0.9) <i>n</i> = 41	0.004 [†]
Triglycerides (mmol/l)	1.1 (0.5) <i>n</i> = 44	1.4 (1.1) <i>n</i> = 114	1.4 (0.6) <i>n</i> = 41	0.11
History of CVD (%)	3.2 <i>n</i> = 62	3.9 <i>n</i> = 129	24.6 <i>n</i> = 65	< 0.001
C-peptide > 0.2 nmol/l during 1 st 5 years (%)	8.9 <i>n</i> = 45	3.8 <i>n</i> = 52	0.0 <i>n</i> = 5	0.57
C-peptide > 0.1 nmol/l during 1 st 5 years (%)	8.9 <i>n</i> = 45	9.8 <i>n</i> = 51	20.0 <i>n</i> = 5	1.0
Heredity hypertension (%)	34.1 <i>n</i> = 44	41.8 <i>n</i> = 98	39.0 <i>n</i> = 41	0.68
Heredity CVD (%)	52.3 <i>n</i> = 44	49.5 <i>n</i> = 97	56.1 <i>n</i> = 41	0.77
Heredity Type 2 diabetes (%)	38.6 <i>n</i> = 44	30.6 <i>n</i> = 98	36.6 <i>n</i> = 41	0.59

Data are means (SD) or (%)

* Significant difference (*p* < 0.05) between all three groups in pairwise comparison

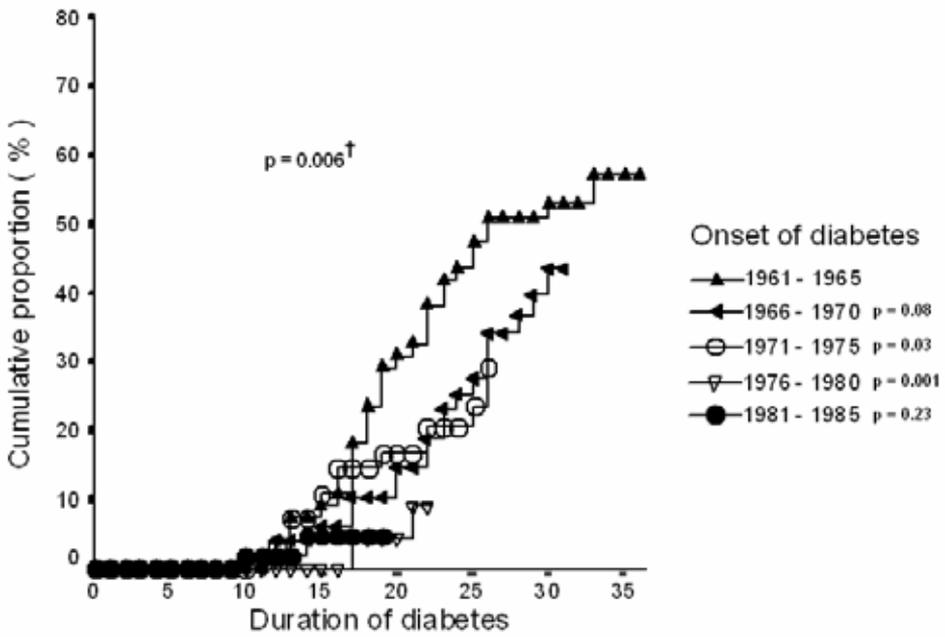
[†] Significant difference (*p* < 0.05) between severe or background retinopathy and no retinopathy

Number of patients with available data differs between risk factors and is indicated by *n*

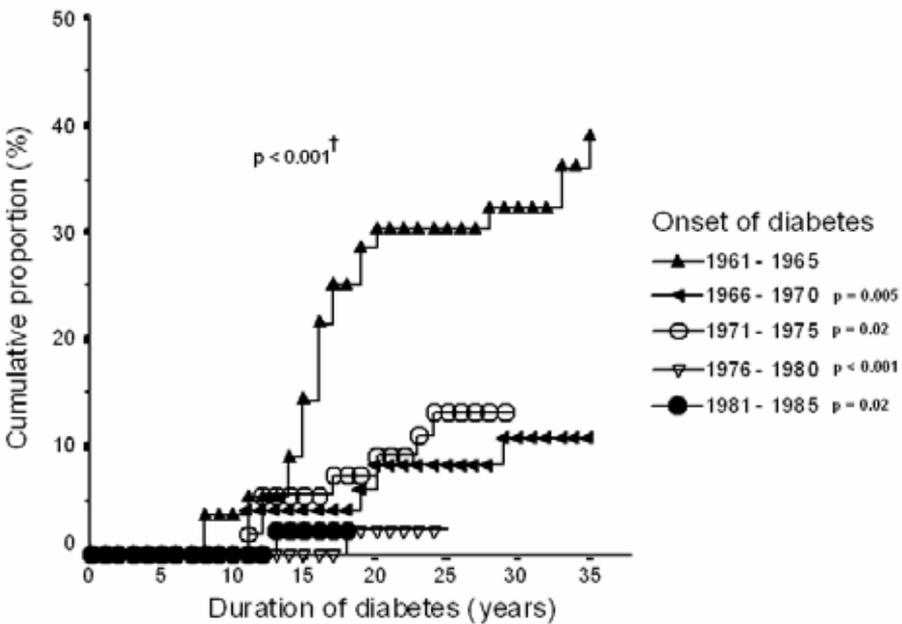
Data for Cpeptide are available only for patients with diabetes onset later than 1975

HbA_{1c} can be transformed to the corresponding DCCT value by adding 1.1%

a)

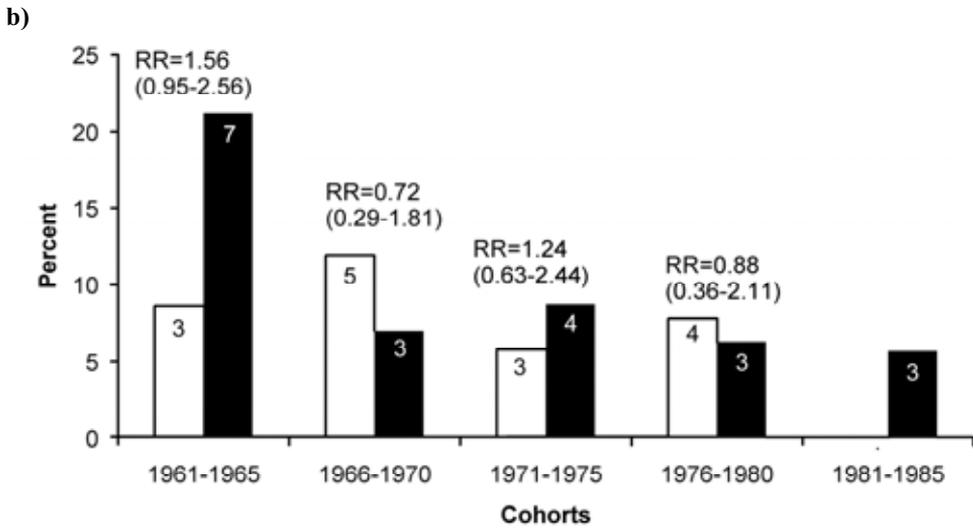
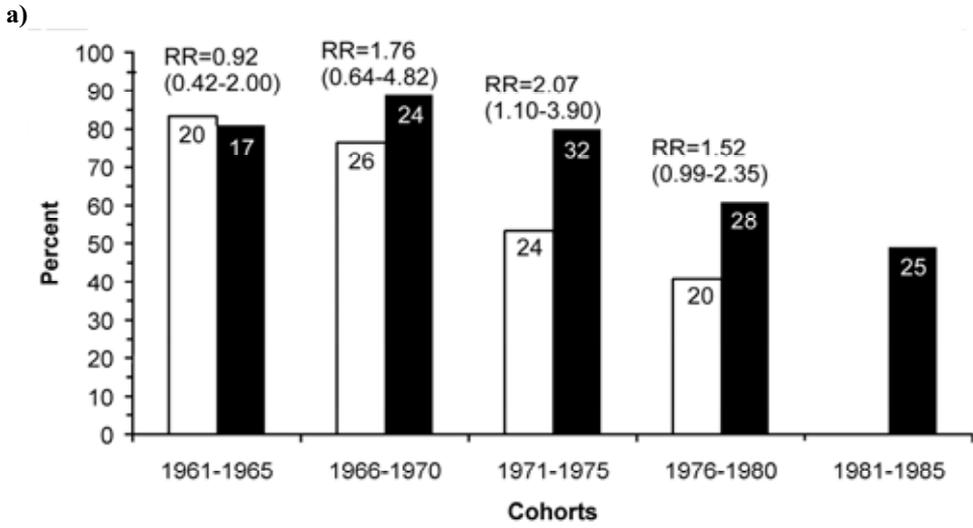


b)



† p-value for overall comparison of all groups. The p-values between the oldest cohort (onset 1961–1965) and the following cohorts are also indicated.

Fig. 4 Cumulative proportion of a) severe (laser treated) retinopathy and b) overt diabetic nephropathy according to the year of onset of diabetes



RR = relative risk with 95% CI
 Number of patients indicated in the bars

Fig 5 Prevalence of a) background retinopathy and b) microalbuminuria in 1990 (□) and at the last follow up in 1997 (■)

The prevalence of background retinopathy was high in all groups, and after 25 years of diabetes duration more than 80% had retinal alterations. The cohort with onset 1971 – 75 deteriorated between 1990 to the last follow up and for the cohort 1976 – 80 the trend was similar (Fig. 5a). The prevalence of microalbuminuria remained at the same level between the two occasions and varied between 6–12% in the patients with diabetes onset 1966 – 1985. In the cohort 1961 – 1965 there was a trend of increasing prevalence from 9% to 21 %, but the difference was not statistically significant (Fig. 5b).

Risk factors for retinopathy and nephropathy

Diabetes duration was longer and HbA_{1c} was significantly higher as well as blood pressure and cholesterol values in the patients with all grades of eye complications at the follow up in the end of the 1990s. The prevalence of CVD and smoking was higher among patients with severe retinopathy. In contrast, BMI, triglycerides, persistence of C-peptide secretion the first five years after diagnosis and heredity for CVD or Type 2 diabetes did not differ significantly between patients with and without eye complications (Table 14). The same pattern was seen among patients with overt nephropathy. However, for patients with microalbuminuria, there was a significant difference compared to patients with no complications only for CVD and smoking, which showed a higher prevalence and for lipids with higher values. There was no difference concerning duration, metabolic control or blood pressure (Table 15).

Table 15 Possible risk factors in patients with Type 1 diabetes diagnosed in childhood with and without nephropathy

Risk factor	No nephropathy n = 210	Microalbuminuria n = 20	Overt nephropathy n = 36	p value
Diabetes duration (years)	24.1 (7.3)	28.0 (7.8)	32.2 (5.6)	< 0.001 [†]
Long term HbA _{1c} (%)	7.2 (0.9) n = 206	7.6 (0.9) n = 19	8.6 (1.1) n = 19	< 0.001 [#]
Systolic blood pressure (mm Hg)	123.3 (8.9) n = 205	123.8 (9.0) n = 19	128.0 (6.3) n = 31	0.02 [†]
Diastolic blood pressure (mm Hg)	76.1 (5.1) n = 205	77.8 (4.6) n = 19	80.7 (6.5) n = 31	< 0.001 [†]
History of smoking (%)	40.3 n = 149	68.8 n = 16	72.2 n = 18	0.006
BMI (kg/m ²)	25.3 (3.5) n = 197	25.6 (3.5) n = 19	27.1 (4.5) n = 25	0.07
Total cholesterol (mmol/l)	4.8 (0.9) n = 163	5.5 (1.4) n = 15	5.5 (1.0) n = 21	< 0.001*
Triglycerides (mmol/l)	1.2 (0.8) n = 163	2.0 (1.6) n = 15	1.6 (0.5) n = 21	0.001 ^{††}
History of CVD (%)	3.9 n = 206	20.0 n = 20	36.7 n = 30	< 0.001
C-peptide > 0.2 nmol/l during 1 st 5 years (%)	5.3 n = 94	16.7 n = 6	0.0 n = 2	0.40
C-peptide > 0.1 nmol/l during 1 st 5 years (%)	9.7 n = 93	16.7 n = 6	0.0 n = 2	1.0
Heredity hypertension (%)	40.9 n = 149	31.3 n = 16	33.3 n = 18	0.65
Heredity CVD (%)	53.4 n = 148	37.5 n = 16	50.0 n = 18	0.48
Heredity Type 2 diabetes (%)	35.6 n = 149	31.3 n = 16	22.2 n = 18	0.51

Data are means (± SD) or %.

[†] Significant difference (p < 0.05) between overt nephropathy and no nephropathy

*Significant difference (p < 0.05) between overt nephropathy/microalbuminuria and no nephropathy

[#] Significant differences (p < 0.05) between overt nephropathy and mikroalbuminuria and no nephropathy, respectively

^{††} Significant difference between microalbuminuria and no nephropathy

Number of patients with available data differs between risk factors and is indicated by n

Data for C-peptide are available only for patients with diabetes onset later than 1975

HbA_{1c} can be transformed to the corresponding DCCT value by adding 1.1%

Table 16 Risk factors for any retinopathy and any nephropathy after adjustment for metabolic control (HbA_{1c}) and diabetes duration

Risk factor	Any retinopathy		Any nephropathy	
	OR (95% CI)	p value	OR (95% CI)	p value
Systolic blood pressure (mm Hg)	1.06 (1.01-1.11)	0.02	1.02 (0.97-1.06)	0.48
Diastolic blood pressure (mm Hg)	1.10 (1.02-1.18)	0.02	1.09 (1.01-1.18)	0.03
History of smoking	0.61 (0.25-1.50)	0.28	1.82 (0.73-4.53)	0.20
BMI (kg/m ²)	1.0 (0.9-1.2)	0.55	1.0 (0.9-1.2)	0.51
Total cholesterol (mmol/l)	1.27 (0.77-2.08)	0.35	1.68 (1.10-2.56)	0.02
Triglycerides (mmol/l)	1.06 (0.60-1.87)	0.84	1.5 (1.00-2.26)	0.05
History of CVD	0.28 (0.04-1.9)	0.19	3.06 (0.91-10.34)	0.07
C-peptide > 0.2 nmol/l during 1 st 5 years of diabetes	2.14 (0.28-16.18)	0.46	28.8 (1.28-649.45)	0.03
C-peptide > 0.1 nmol/l during 1 st 5 years of diabetes	3.79 (0.67-21.35)	0.13	1.76 (0.17-18.75)	0.64
Heredit hypertension	1.50 (0.63-3.54)	0.36	0.88 (0.35-2.20)	0.79
Heredit CVD	0.66 (0.28-1.56)	0.34	0.56 (0.23-1.36)	0.20
Heredit Type 2 diabetes	0.61 (0.25-1.45)	0.26	0.73 (0.28-1.90)	0.51

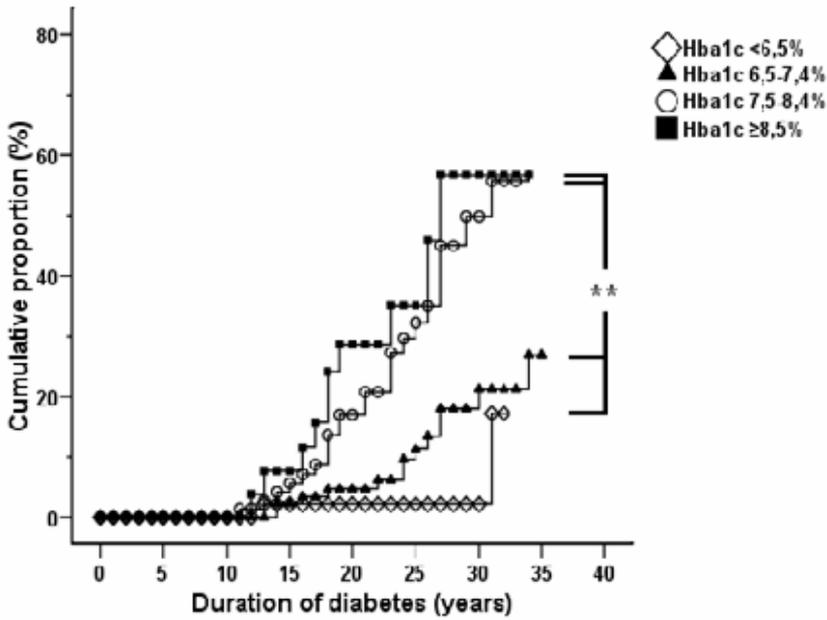
Odds ratio (OR) for dichotomous variables has as reference group those patients without the respective factor

The relation between possible risk factors and prevalence of any retinopathy and any nephropathy at follow up was analysed with the use of logistic regressions models, adjusting for the possible confounding effects of metabolic control and diabetes duration (Table 16). Of the univariate associations only systolic and diastolic blood pressure were still significant risk factors concerning retinopathy. Diastolic blood pressure and lipids were the only significant risk factors concerning nephropathy. Even these relations were abolished when all possible risk factors were tested in a multivariable model. Only diabetes duration OR (95% CI) 1.2 (1.1–1.3), $p < 0.001$ and HbA_{1c} OR (95% CI) 4.1 (1.8–9.2), $p = 0.001$ showed a significant correlation to any retinopathy. All other factors were insignificant. The same pattern was seen concerning any nephropathy, where diabetes duration OR (95% CI) 1.1 (1.0–1.2), $p = 0.016$ and HbA_{1c} OR (95% CI) 2.6 (1.3 – 5.1), $p = 0.007$ showed a significant association.

Metabolic control

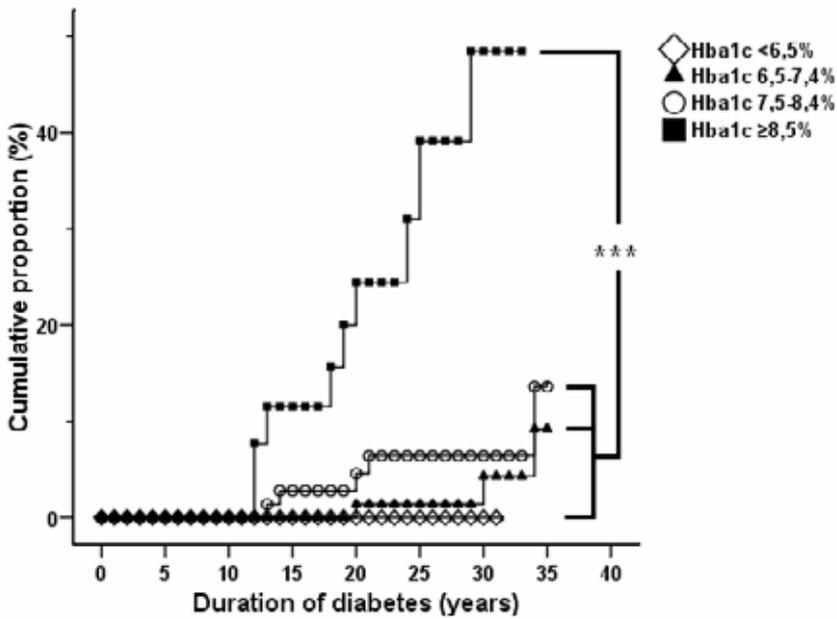
The cumulative proportion of severe retinopathy and overt nephropathy for patients with different long term HbA_{1c} is illustrated in Fig 6a and b. The risk of retinopathy is increasing significantly if long term HbA_{1c} is higher than 7.5%. In contrast, the risk of overt nephropathy is increasing significantly first when HbA_{1c} values increase above 8.5%.

a)



$p < 0.001$ for overall comparison of groups. In pairwise comparison $p < 0.01$ between groups indicated in the figure **.

b)



$p < 0.001$ for overall comparison of groups. In pairwise comparison $p < 0.001$ between groups indicated in the figure ***.

Fig. 6 Cumulative proportion of a) severe retinopathy and b) overt nephropathy according to long term metabolic control

Diabetic complications in patients with intensive insulin treatment from onset of diabetes

The prevalence of subclinical neuropathy was high, noted already at the first examination after mean diabetes duration of 8.1 years. It increased from the first to the second examination, but 4 patients improved and had normal results at follow up. In total 60% showed abnormal nerve conduction on at least one occasion. The prevalence of retinopathy was also relatively high, 28%, but there were just a few cases of more severe forms. On the contrary, the prevalence of nephropathy was low, 5%, and just one patient had macroalbuminuria (Table 17).

Only 24 (30%) did not have any complications at all. Neuropathy was not a significant risk marker for the other complications, even if there was a trend for higher prevalence of both nephropathy and retinopathy among the patients with neuropathy. Thirty-two (68%) of the patients with neuropathy had no other complications and 8 (33%) of the patients with retinopathy or nephropathy had no signs of neuropathy.

Mean HbA_{1c} for the whole population was 7.3% (range 5.2 – 11.4%) and differed between subgroups of patients. The patients with complications had higher HbA_{1c} than the patients without complications, but the difference was not statistically significant for the patients with

Table 17 Prevalence of subclinical neuropathy, retinopathy and nephropathy and metabolic control in a population of young patients with intensively treated Type 1 diabetes

	n	%	HbA _{1c}		Duration (years)		
			%	p value	Mean(SD)	range	p value
Nerve function							
Normal	32		6.9 (0.8)	0.001 [¶]	11.4 (3.2)	7.1-18.9	0.7 [¶]
Neuropathy	47		7.6 (1.0)		11.7 (3.8)	4.5-20.6	
Examination 1	29	39.2			8.1 (3.4)	3.7-17.4	
Examination 2	39	55.7			11.9 (3.7)	7.4-20.6	
Examination 1 or 2	47	59.5			11.6 (3.8)	4.5-20.6	
Eye examination							
Normal	58		7.2 (0.9)	0.04 [¶]	12.5 (3.5)	8.2-24.5	<0.001 [¶]
Retinopathy	22	27.5	7.7 (1.1)		15.8 (3.9)	9.9-22.8	
Background	18	22.5			15.7 (3.8)	10.7-22.8	
Preproliferative/ Proliferative	4	5.1			16.0 (4.7)	9.9-21.5	
Kidney examination							
Normal	75		7.3 (1.0)	0.3 [¶]	13.2 (3.7)	7.5-22.7	0.9 [¶]
Nephropathy	4	5.1	7.8 (0.9)		12.9 (3.5)	8.9-16.8	
Microalbuminuria	3	3.8			11.5 (2.8)	8.9-14.6	
Macroalbuminuria	1	1.3			16.8		
All complications							
None	24		6.8 (0.8)	0.004 [†]			
Only neuropathy	32		7.5 (0.8)				
Retinopathy or nephropathy	24		7.6 (1.1)				

HbA_{1c} values are mean value of yearly mean for all individuals (SD). HbA_{1c} can be transformed to corresponding DCCT value by adding 1.1%

[¶] p-value for comparison between patients with and without complications

[†] p-value for over all comparison. In pairwise comparisons the p-value was 0.02 between no complications and only neuropathy, 0.006 between no complications and retinopathy/nephropathy and 1.0 between only neuropathy and retinopathy/nephropathy.

Table 18 Clinical characteristic of patients at onset of Type 1 diabetes diagnosed in childhood 1976–2000

Period of onset	1976–80	1981–85	1986–90	1991–95	1996–2000	All periods
<i>n</i>	51	56	53	66	90	316
Duration of symptoms prior to diagnosis weeks (SD)	2.9 (2.4)	2.6 (2.8)	2.7 (2.2)	2.6 (3.3)	2.8 (2.9)	2.7 (2.8)
DKA at onset (%)	15.7	10.7	13.5	20.3	18.9	16.3
HbA _{1c} at onset % (95% CI)	9.4 (8.2-10.6) <i>n</i> = 11	8.9 (8.3-9.4) <i>n</i> = 51	8.6 (8.0-9.2) <i>n</i> = 44	9.3 (8.6-10.0) <i>n</i> = 52	9.3 (8.8-9.7) <i>n</i> = 87	9.1 (8.8-9.3) <i>n</i> = 245
Prevalence of partial remission (%)	68.6	64.3	62.3	63.6	43.3 *	58.5

n = number of patients with available data DKA = diabetic ketoacidosis * *p* < 0.01 compared to the other groups

nephropathy. The patients with only neuropathy did not differ from the patients with other forms of complications (Table 17).

The duration of diabetes was the same among the patients with and without complications besides the patients with retinopathy who had longer diabetes duration (Table 17).

Clinical characteristics of patients at onset of diabetes during 25 years

Age at onset, gender and onset before puberty didn't differ significantly between the cohorts with diabetes onset during the time period 1976 – 2000. The duration of symptoms prior to diagnosis, prevalence of DKA and HbA_{1c} at diagnosis was also the same in all cohorts (Table 11 and 18).

Secular trend of diabetes partial remission and C-peptide secretion

The prevalence of partial remission during the whole time period was 59%. The youngest cohort 1996 – 2000 had a significantly lower rate. Between the other cohorts there was no difference (Table 18). The median (range) duration of partial remission for the patients with a remission period was 0.9 (0.1–6.2) years and there was no difference between cohorts (Fig 7).

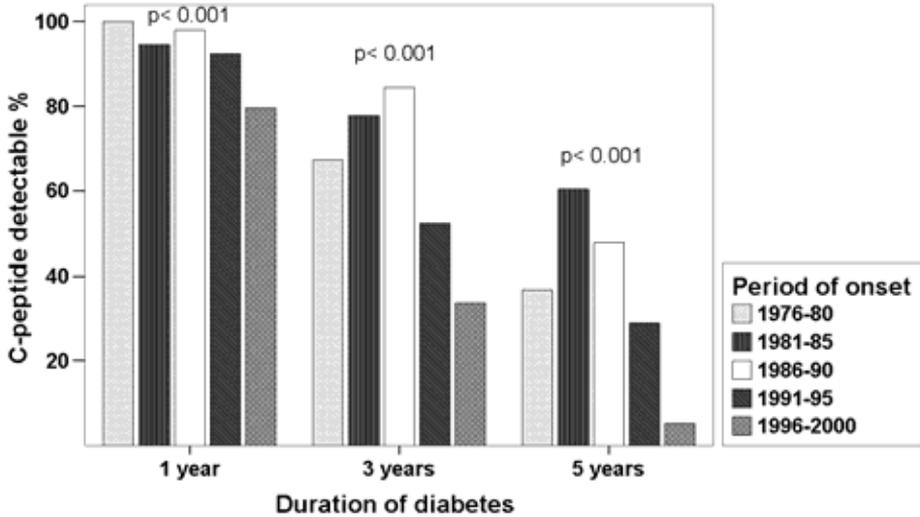


p = 0.22 for comparisons of groups.

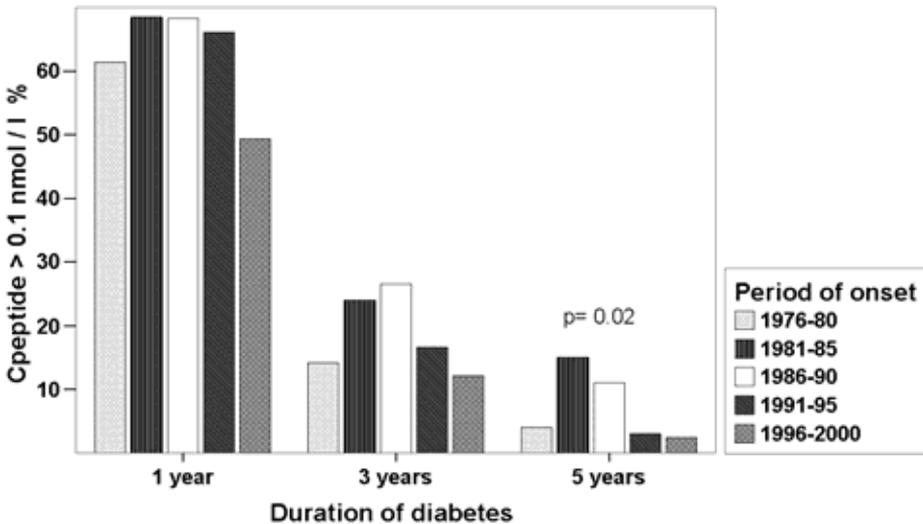
Fig. 7 Duration of partial remission for patients with a remission period

The prevalence of detectable fasting C-peptide was 91.2% in the whole population after 1 year of diabetes duration and significantly lower in the cohort 1976 – 2000. After 5 years still 32.7% of the patients had detectable fasting C-peptide (Fig. 8a). If the cut off level was increased the prevalence was lower, 61.2% had C-peptide > 0.1 nmol/l after 1 year of diabetes, decreasing to 6.5% after 5 years (Fig. 8b). There was a significant difference between cohorts concerning detectable C-peptide, with the cohort 1996 – 2000 having the lowest prevalence at all durations. The same cohort also had the lowest prevalence of C-peptide > 0.1 nmol/l after 5 years. All other comparisons among cohorts were non significant.

a)



b)



Significant differences between cohorts in overall comparison indicated by p value in the figure

Fig. 8 Prevalence of fasting serum-C-peptide after 1, 3 and 5 years of diabetes duration
a) detectable C-peptide ≥ 0.03 nmol/l. b) C-peptide > 0.1 nmol/l

C-peptide secretion, both fasting and stimulated maximum value, was highest after 3 months of diabetes duration and then declined successively (Fig. 9). The three latest cohorts had higher C-peptide values at onset ($p < 0.05$) and maximum value was significantly higher ($p < 0.05$) at 3 months in the cohort 1996 – 2000 compared to the two earliest cohorts. In contrast, fasting C-peptide was lower in the cohort with diabetes diagnosis in 1996 – 2000 ($p < 0.05$) after 1.5, 2.5 and 4 years. All other pairwise comparisons were non significant.

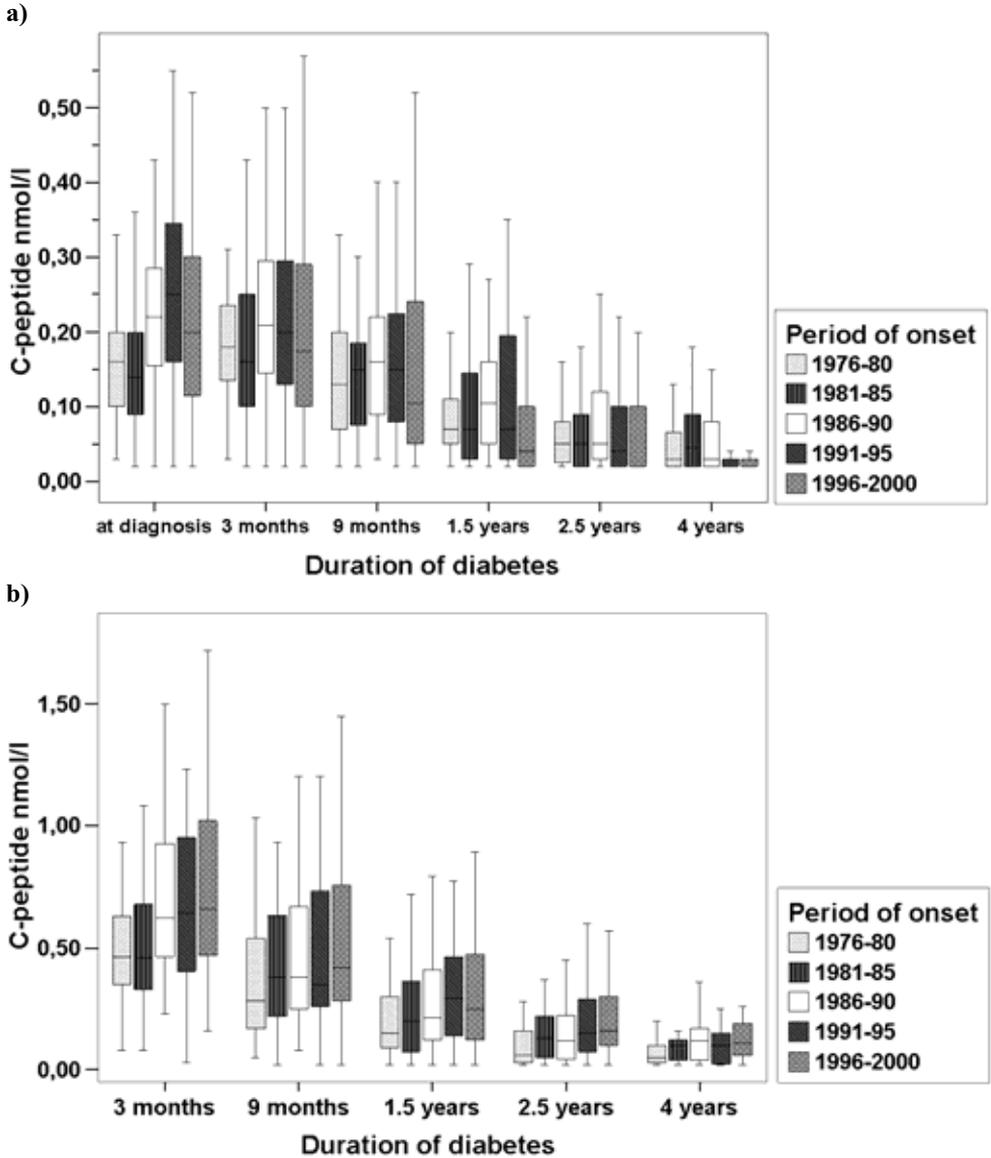
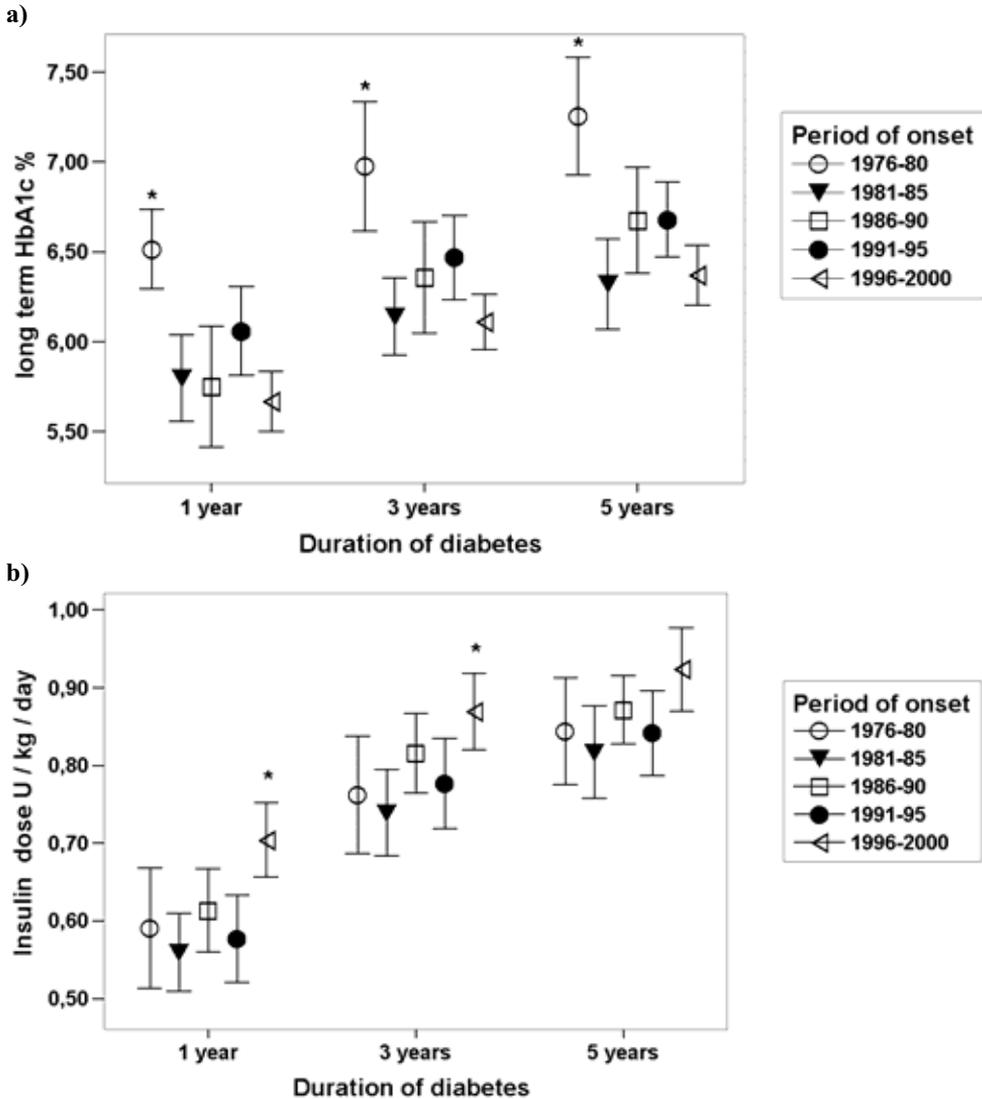


Fig. 9 C-peptide secretion after different diabetes duration. a) Serum-C-peptide at diagnosis and fasting serum-C-peptide b) Maximum C-peptide after stimulation with a mixed meal

C-peptide secretion and metabolic control

Long term HbA_{1c} was higher for the cohort 1976 – 1980 during all years. The insulin dose was higher for the cohort 1996 – 2000 during 1 and 3 years of diabetes duration. During the whole 5 years period there was no difference between cohorts (Fig. 10). Both HbA_{1c} and insulin dose were significantly lower in patients with persistent C-peptide secretion > 0.1 nmol/l during 1, 3 and 5 years (Fig. 11). If "detectable" C-peptide was used as cut off level it was still possible to demonstrate a significant difference between groups concerning insulin dose, but not concerning HbA_{1c} after more than 1 year of diabetes duration (data not shown).

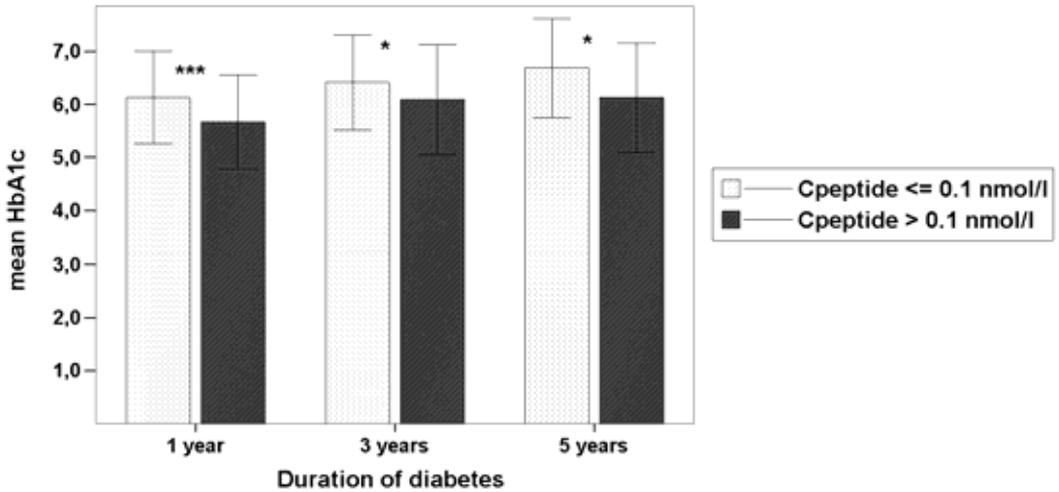


Error bar represent mean value and 95% CI

* indicate cohort, which significantly ($p < 0.05$) differs from the other cohorts at the respective diabetes duration.

Fig. 10 a) Long term HbA_{1c} and b) insulin doses in patients with Type 1 diabetes diagnosed 1976 – 2000 during 1, 3 and 5 years of diabetes duration

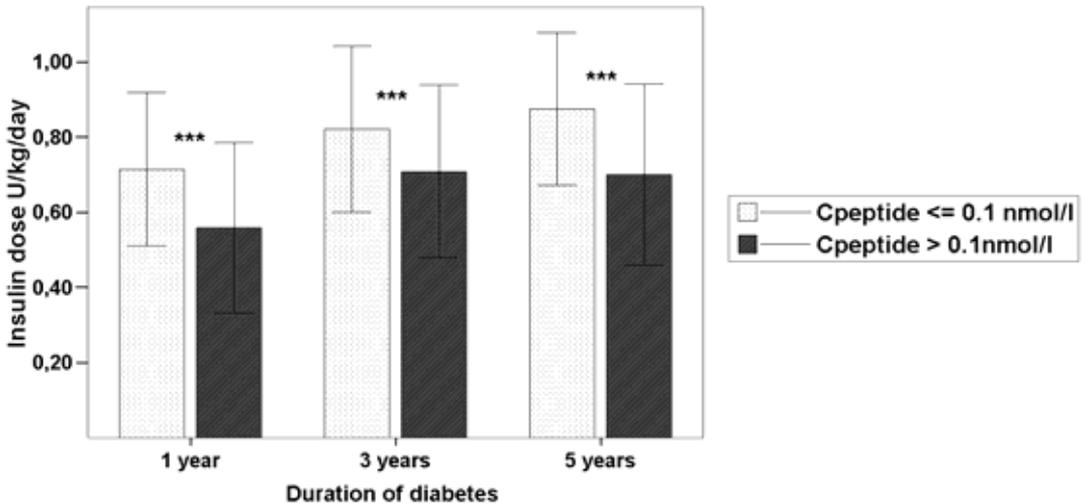
a)



Bars show mean HbA_{1c} and error bars SD.

* $p < 0.05$ *** $p < 0.001$ for difference between groups.

b)



Bars show mean insulin dose and error bars SD.

*** $p < 0.001$ for difference between groups.

Fig. 11 a) HbA_{1c} and b) insulin dose during the first five years after diabetes diagnosis in relationship to prevalence of fasting C-peptide > 0.1 nmol/l

In a logistic regression model with C-peptide > 0.1 nmol/l after 5 years as dependent variable, the influence of sex, age at diagnosis, HbA_{1c} at diagnosis, DKA at diagnosis, C-peptide at diagnosis and long term HbA_{1c} and insulin dose during the first five years were analysed. The most obvious statistically significant associations was age at diagnosis, OR (95% CI) = 2.0 (1.3 – 3.1) $p = 0.001$; HbA_{1c} at diagnosis, OR = 1.8 (1.2 – 2.8) $p = 0.005$ and insulin dose, OR = 0.002 (0 – 0.2) $p = 0.005$. But even male gender OR = 0.1 (0.02 – 0.6) $p = 0.014$ and DKA at diagnosis OR = 0.03 (0.02 – 0.6) $p = 0.02$ showed a significant association.

Table 19 Mortality in a population of patients with Type 1 diabetes diagnosed in childhood during 1961 – 1985

Period of onset	<i>n</i>	Uraemia	CHD ^a	Hypo-glycaemia	Suicide	Keto-acidosis	Accident	Cancer	All causes
		<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>
1961-1965	56	6	4 ^b				1 ^c	1	12
1966-1970	50	1	1	1	2				5
1971-1975	55		1 ^c	2		1 ^c			4
1976-1980	51			1		1			2
1981-1985	57				1	1			2
All periods	269	7	6	4	3	3	1	1	25

^a CHD Coronary heart disease ^b2 of the patients had diabetic nephropathy

^c The patient had diabetic nephropathy

Mortality

The mortality rate was high in the oldest cohort (Table 19). Of the 25 deaths, 7 were directly caused by nephropathy and uraemia. The mortality was highest among the patients with nephropathy, 12 (33%) of 36 patients compared with 13 (6%) of 230 patients without nephropathy.

Discussion

In this unselected population with Type 1 diabetes diagnosed in childhood during the years 1961 – 1985 the cumulative proportion of overt nephropathy and severe retinopathy after 30 years of diabetes duration had declined during the last decades. Diabetes duration and long term HbA_{1c} were the only significant risk factors for development of both retinopathy and nephropathy. Early microvascular complications were still common in a population of young adults with mean 13 years of diabetes duration, despite fairly good metabolic control and intensive insulin regimens from onset of diabetes. The prevalence of subclinical neuropathy was high and all grades of retinopathy relatively high, but lower than usually reported. On the contrary, the prevalence of microalbuminuria was low. The clinical characteristics at onset of diabetes have remained unchanged over the last 25 years despite the doubling of diabetes incidence and more intensive insulin regimens from beginning. The duration of partial diabetes remission and magnitude and duration of C-peptide secretion have also been unchanged.

Study population

We studied an unselected population of Type 1 diabetes in a well defined geographic area, since every child in Sweden with newly diagnosed diabetes is treated at a paediatric clinic. The patients were treated in routine care. We managed to trace nearly all the patients and had very few drop-outs. Of the 269 patients in study I and IV, 91 % were monitored for retinopathy and 95% for nephropathy until at least 1997. In study II, 79 of 80 patients participated in at least one electrophysiological examination of nerve conduction velocity and all patients were monitored for nephropathy or retinopathy. This makes the results reliable and possible to apply in clinical practice. In study III the participant rate varied between 81 – 93% on different occasions. The drop-outs were even distributed between cohorts and we have no reason to believe that they were biased with time. Because of difficulties with repeated blood sampling in preschool children, they are to a lesser extent represented in the results of stimulated C-peptide secretion, but the proportion of younger children was alike in the different cohorts.

Statistical methods

Survival analysis was used for investigation of cumulative proportion of severe retinopathy and overt nephropathy. This method requires exact time for the events, but even information from patients not monitored until the end of the study period can contribute to the calculations. That means a better possibility to discover differences between groups of patients. The cumulative proportion (or probability) calculated with life table method is mathematically not the same as cumulative incidence, but if there are not too many drop outs the figures are roughly comparable.

The results of cross-sectional prevalence studies are difficult to compare with our study, since prevalence studies tend to give lower figures after long term follow up of severe complications as a considerable proportion of the patients with overt nephropathy die prematurely. However, we were also forced to use cross-sectional data for some variables. Background retinopathy and microalbuminuria are sometimes reversible, less well defined and were not regularly measured during the whole study period, which makes it inappropriate to use survival analysis. We used instead prevalence rate for these complications. The same is valid for subclinical neuropathy, which was not screened for in the clinical routine.

Only intervention studies can give a definite answer whether associations between risk factors are causal. In this type of observational study, there is a complex interrelationship between different risk factors and it is necessary to use statistical methods such as logistic regression models and Cox proportional hazard analysis to adjust for confounding factors. However, introduction of several variables in the analysis can diminish the possibility to really discover significant associations, especially if the population is quite small as in our study. We can't exclude that lack of association in some cases could partly be explained by too low statistical power.

Information about smoking and hereditary factors was collected from a questionnaire sent to the patients in the end of the 1990s. The responding rate of 68% in the whole study population was acceptable and at the same level as in other comparable studies. However, the responding rate was slightly lower in the two oldest cohorts, partly because of the higher mortality in overt nephropathy in these cohorts. That means that data is lacking for a rather great part of the patients with severe complications and the same is true concerning HbA_{1c} values, since HbA_{1c} values were not available for 47% of the patients with overt nephropathy and for 25% of the patients with severe retinopathy, who developed complications before the method was introduced in 1980. These circumstances can diminish the power of the statistical analysis. In spite of this, we found a strong association between metabolic control and microvascular complications.

Definitions of long term complications

The long follow up time in study I and IV means difficulties, since laboratory methods, examination procedures and definition of complications could have changed during the period. We defined severe retinopathy as laser treated retinopathy as it is more definite than just proliferative retinopathy, especially in the early period when the eyes were examined with ophthalmoscopy. It is possible that the indications have changed into a more liberal direction the last years. However, this would tend to overestimate the cumulative proportion in the younger cohorts compared with the older ones. In study II and IV all grades of retinal changes were included, but at that time all patients were examined with fundus photographs. Even if the photos were examined by different ophthalmologists, there is a general consensus about the classifications of retinopathy (Table 5). Other studies from the same region have shown a good agreement between the ophthalmologists⁹¹.

Overt nephropathy was defined as persistent macroproteinuria. During the first decades it was measured only with semi-quantitative test strips and there are several sources of error. The test strips are not well validated. When the metabolic control is bad the urine is diluted and the protein concentration false low. However, the definition is the same as in other older studies, which makes it possible to compare the results. The prevalence of microalbuminuria was defined as AER 20 – 200 µg/minute in a timed over night urine sample, which is a general accepted definition, at the last available visit. We accepted a spot value to classify the patient as microalbuminuric, which probably have overestimated the prevalence. On the contrary, the use of ACE inhibitors and other antihypertensive medicines could reduce the prevalence of microalbuminuria. In study I 25 patients were prescribed antihypertensive medicine before the occurrence of nephropathy. The great majority of these patients (22) belonged to the cohorts 1961 – 65 and 1966 – 70 and it is possible that this to some extent has decreased the prevalence at the last follow up in these cohorts, but it can not explain the low prevalence of microalbuminuria in the youngest cohorts. In study II no patient used that sort of medicine.

Laboratory methods

The HbA_{1c} method has changed several times during the follow up period, but the laboratory had conversion equations every time the method was changed (Table 12). The same method was used during 10 years (1986 – 1996) and during the last years it was continuously controlled against a reference method. Even laboratories outside the region could give us conversion factors to the actual reference method, making it possible to use data from patients who moved. We do not have any reason to believe that there is a systematic error between HbA_{1c} values from different time periods. On the other hand the CV% was larger for the older methods, which tends to make it more difficult to find differences between groups. Despite this, we found a clear significant effect of metabolic control on the risk of development of complications. The HbA_{1c} methods differ between laboratories world wide, making it difficult to compare results from studies in other countries. However, a previous study from our laboratory has shown that the actual Swedish reference value is 1.1% lower than the corresponding DCCT-value, making comparisons in many cases possible²⁸¹. In study III and IV we used mean HbA_{1c}, weighted for the time between the measurements²⁸², as a measure of long term metabolic control. This is a more appropriate measure than just the mean of HbA_{1c} as this method can correct for varying time intervals between measurements. Otherwise periods with very frequent analysis of HbA_{1c}, for example during periods with bad metabolic control and during pregnancy with very good metabolic control, can influence the mean in an incorrect way.

Serum-C-peptide analysis was performed by the same method during most of the study period and by the same technician during 18 years, which implies that the values ought to be comparable between cohorts. However, after 2001 the method was changed and the blood samples were stored in the freezer for a longer time period before analysis. We can not exclude that this has influenced the results for the last cohort with false low values. The decreasing C-peptide values at follow up in this cohort could possibly be explained by these technical reasons, which is why we have been very cautious in our interpretation of this part of the study.

Incidence and prevalence of long term complications

The cumulative proportion of severe retinopathy was more than 50% after 30 years of diabetes duration in the cohort with diabetes diagnosis 1961 – 65, which is similar to other studies in the 1980s^{39, 89}. The cumulative proportion then declined significantly in overall comparison between cohorts and the difference was statistically significant between the cohort 1961 – 65 and the cohorts 1971 – 75 and 1976 – 80. To our knowledge this has not been previously reported in an unselected population with diabetes beginning in childhood. A study from Denmark reported in 2000 a declining trend of proliferative retinopathy with increasing calendar year of diagnosis, but the patients were older at diagnosis and the study was clinic-based. The decrease appeared about 10 years later compared with our population and was noted first for the cohort with diabetes diagnosis 1979 – 84⁷⁵. Long term metabolic control seemed to be worse for the oldest patients in the Danish study compared to our patients, which possibly could explain the difference observed. After the method was introduced in the early 1980s long term HbA_{1c} was 8.8 – 8.9% in the Danish cohorts with onset of diabetes 1965 – 1979, while the cohort 1979 – 1984 had a lower HbA_{1c} of 8.5%. In comparison, already for the cohorts 1966 – 1980, long term HbA_{1c} in our patients was 8.4 – 8.5% (DCCT-corrected value) (Table 11).

The prevalence of non-proliferative retinopathy in study I was high in all cohorts and increased between 1990 and 1997 in accordance with older studies, which reported that

almost every patient had developed background retinopathy after 20 – 30 years of diabetes duration^{39, 89}. The cumulative proportion of severe retinopathy continued to rise during the whole observation period for all cohorts. This speaks in favour of that we have just postponed severe retinopathy. On the contrary, in study II we found a prevalence of any retinopathy of 27% and very few cases of more severe forms. That seems to be lower than in many other comparable studies, in which the prevalence after similar duration was 40 – 65%^{39, 54, 55, 85, 166}. In our study the prevalence was at the same level as in a recent study from the whole south east region in Sweden, where the prevalence was 32% after 10 – 12 years of diabetes duration⁹¹. The clinical importance and prognostic significance of background retinopathy in a longer time perspective is unclear. Milder forms are reversible^{81, 284}, even if a higher count of microaneurysms is predictive of higher rate of proliferative retinopathy the next years⁸². Longer follow up is necessary before we can draw conclusions about whether it is possible to prevent even severe retinopathy.

The cumulative proportion of overt nephropathy of 32% after 30 years of diabetes duration in the oldest cohort with diabetes diagnosis 1961 – 65 is comparable with other studies from the 1980s and early 1990s^{30, 39, 70, 73}. For the following cohorts we found a markedly reduced cumulative proportion of only 8% and 13%, respectively, after 25 years of diabetes duration, remaining largely unchanged after 30 years of diabetes duration. In the above mentioned study from Denmark⁷⁵, they also found a declining trend during the last decades, but again not before the cohort with diabetes diagnosis 1979 – 84, i.e. 15 years later than in our population. During the last years some prevalence studies, the Eurodiab Study and a study from Wales, also have reported a declining trend to about 20% after 20 – 30 years of diabetes duration^{56, 76}. That is still much higher than in our population.

We found a low and constant prevalence of microalbuminuria in study I and a very low prevalence in study II of only 4%, despite using a spot value for classification. This is clearly lower compared with other studies from recent years, where a prevalence of 10–15% is common^{52, 54, 55, 92}. Microalbuminuria is a risk factor for development of overt nephropathy and about 20% will progress to macroalbuminuria within 7 – 10 years⁶³⁻⁶⁸. The progression rate would probably be even lower in adolescents⁶⁸. The low prevalence of microalbuminuria in our study population and the largely unchanged cumulative proportion of overt nephropathy after 25 to 30 years of diabetes duration speaks in favour of that nephropathy has really been prevented and not just postponed.

We found a high prevalence of subclinical neuropathy of nearly 60% in accordance with many other studies, even if different methods for definition of neuropathy hamper comparisons^{54, 110, 111, 114}. An interesting question is why we still find a high prevalence of subclinical neuropathy, but a declining incidence of other microvascular complications in this population. Subclinical neuropathy was noted after just a few years of diabetes duration as in other studies^{101, 121} and there was no correlation between diabetes duration and occurrence of neuropathy, also reported in other studies^{85, 100, 105, 111, 112}. The patients with signs of subclinical neuropathy had higher HbA_{1c} than patients without neuropathy, but there was no difference between patients with just neuropathy or other types of complications. This argues against the nerves being more sensitive for worse metabolic control. Another explanation is that we used a more sensitive diagnostic method for nerve dysfunction than for the other complications, which possibly could be equally common with other examination methods. With kidney biopsy it is possible to demonstrate changes in the kidney very early after diabetes diagnosis^{173, 174}. Fluorescein angiography can detect incipient forms of retinopathy several years earlier than fundus photography⁸⁴. Our study could not analyse this theory. A

third possibility is that there are different pathogenetic mechanisms causing different kinds of long term complications. Long term metabolic control is certainly not the only factor and more acute effects of hyperglycaemia and other metabolic disturbances could also be of importance²⁸⁵. The weak association between the occurrence of neuropathy and other types of complications and the lack of association with diabetes duration speaks in favour of this explanation.

Risk factors

There is a complex interrelationship between the different possible risk factors for long term complications and the cause and consequences are not always obvious. We found several significant univariate associations completely abolished in the multivariable analysis. It is absolutely necessary to adjust for confounding factors, which is not always done in other studies and makes comparison of results difficult.

Metabolic control In study IV long term HbA_{1c} was, beside diabetes duration, the only highly significant independent risk factor for both diabetic retinopathy and nephropathy, in multivariable models abolishing all other univariate associations of risk factors. Even in study II metabolic control was worse in patients with neuropathy and retinopathy. The inability to find a correlation between nephropathy and metabolic control in this study was probably explained by too low statistical power. The result is in concordance with practically all other population studies and intervention studies as the DCCT, the Oslo and the Stockholm study^{32, 128, 286} over the last decades.

There has been debated whether there is a threshold value for HbA_{1c} and the risk of complications. Our study was not designed to give a definite answer to that question, but the risk for overt nephropathy was very small below HbA_{1c} 8.5% and no patient with HbA_{1c} < 6.5% had nephropathy despite long duration. The risk for severe retinopathy began to increase already when HbA_{1c} increased above 7.5% and there was in our study no “safe” level. However, the recent guidelines from ADA with a recommended level of HbA_{1c} < 6.0% may be unnecessarily strict¹³⁰. The different risk levels of HbA_{1c} for retinopathy and nephropathy suggest a different susceptibility in these organs for the harmful effect of hyperglycaemia.

Duration Not unexpectedly, duration of diabetes was a significant independent risk factor for retinopathy in study II and IV and for nephropathy in study IV, in concordance with practically all other studies. In study II we did not find any difference between patients with and without nephropathy, probably explained by too few cases and low statistical power. We did not find any correlation between neuropathy and diabetes duration, which is also in concordance with other studies. Since blood pressure, BMI, lipid values and prevalence of history of CVD and smoking increase with age, which of course highly correlates with diabetes duration, it is important to always adjust for the effect of duration as a confounding factor. Diabetes duration was therefore included in all multivariable models.

Blood pressure We found a univariate association between both systolic and diastolic blood pressure and overt nephropathy and any retinopathy. After adjusting for diabetes duration and metabolic control the relationship between systolic blood pressure and overt nephropathy disappeared. The other associations were weak but statistically significant, but in multivariable analysis even these associations were abolished. Many studies have convincingly demonstrated the valuable effect of antihypertensive therapy to retard the progress of renal failure^{77, 175}. On the contrary, there are conflicting results concerning the importance of blood pressure level as a risk factor for the initial development of diabetic

retinopathy and nephropathy^{54, 65, 103, 117, 138, 163, 170}, perhaps partly explained by not adjusting for the confounding effect of metabolic control, diabetes duration and early stages of nephropathy.

We analysed in this study the association between long term blood pressure and diabetic complications. It is possible that this may have underestimated the importance of higher blood pressure values during a shorter time period for the development of complications. On the other hand, if only the last year's blood pressure is taken in consideration there is a risk of overestimating the causal effect of higher blood pressure values. Renal structural abnormalities are present long before they are possible to detect with usual laboratory test^{173, 174}. Twenty four- hour ambulatory blood pressure is pathologic already with AER within upper normal range¹⁷² and higher nocturnal mean arterial blood pressure was in normoalbuminuric children associated with more advanced renal changes¹⁰⁶. A higher blood pressure could thus be a secondary phenomenon to an early kidney disease. This population was too small to allow for dividing the blood pressure values in shorter time periods and perform subgroups analysis. This discussion is valid not only for nephropathy, but even for diabetic retinopathy, since many of these patients have concomitant renal disease¹⁷⁰.

Our study does not support the importance of higher blood pressure levels for the development of microvascular complications. However, the role of blood pressure levels must still be considered unclear and intervention studies with early antihypertensive therapy and long follow up are necessary to eventually investigate the clinical importance and create guidelines for clinical practice.

Smoking We found a significant univariate association between smoking and severe retinopathy and any nephropathy. But after adjusting for diabetes duration and metabolic control the associations were completely abolished. Diabetes duration is probably a confounding factor explained by a cohort phenomenon. History of smoking was more prevalent in the oldest cohorts with longest diabetes duration and decreased from 70 % to 30 % in the youngest cohorts. This is in concordance with the markedly changed smoking habits in Sweden the last decades (Official statistics, The National Board of Health and Welfare in Sweden). Metabolic control can also be a confounding factor with psychosocial factors affecting both smoking habits and the ability to achieve good metabolic control¹⁹⁰, even if some authors have suggested a direct effect of smoking on metabolic control and insulin sensitivity²⁸⁷. Other studies have given conflicting evidence of the connection between smoking and diabetic complications^{65, 163, 194, 195}, but in our study the higher incidence of complications in the oldest cohorts seems not be explained by smoking habits.

Other cardiovascular risk factors BMI was the same in all cohorts and did not differ between patients with and without complications. We measured BMI at follow up in the end of the 1990s and we can not exclude that the results would have been different with long term BMI. However, this is in concordance with some studies^{167, 288}, while others have found BMI to be a risk factor for both retinopathy and nephropathy^{117, 162, 289}. Dyslipidemia is also considered part of the metabolic syndrome, which is suggested to be a risk factor for diabetic complications¹⁶⁰. In univariate analysis we found higher cholesterol values in patients with both any retinopathy and any nephropathy and higher triglycerides in patients with nephropathy. After adjusting for diabetes duration and metabolic control the association remained only for any nephropathy. The causal relationship between dyslipidemia and diabetic nephropathy is impossible to investigate in this type of observational study and intervention studies are required to further analyse the importance for the initial development

of complications. In multivariable regression models the association was abolished, again emphasizing the importance of metabolic control. Heredity for CVD, Type 2 diabetes and hypertension did not differ between patients with and without complications and our study can not support the theory of inheritance of the metabolic syndrome explaining the genetic susceptibility for long term complications²⁹⁰. History of CVD was more common among patients with complications, as demonstrated in many other studies. Again, adjusting for diabetes duration and metabolic control abolished the relationship. In our study we can not further analyse if there is a common pathogenetic factor for CVD and nephropathy, suggested by some authors⁴² or if the higher prevalence of CVD in patient with nephropathy are secondary to the higher frequency of hypertension and dyslipidemia in these patients^{41,43}.

C-peptide The proportion of patients with persistent C-peptide secretion the first five years after diabetes diagnosis was the same in patients with and without retinopathy and nephropathy. There are few population studies in this field and no one could demonstrate an effect of C-peptide secretion when adjusting for metabolic control^{255-261, 291}. In the DCCT study the patients with C-peptide secretion had a slower rate of progression of microvascular complications, mostly explained by a better metabolic control²⁴², but the follow up was shorter than in our study. However, it seems reasonable that the effect of C-peptide secretion during the first years diminishes in importance after a longer follow up, since the C-peptide secretion gradually decreases during the years after diabetes diagnosis.

In this population we found good glycaemic control to be the most crucial factor for prevention of long term complications. We could not confirm the importance of long term blood pressure, lipid profiles, BMI and smoking demonstrated in some other studies. We can not exclude that the explanation to some extent can be too low statistical power in this rather small population. However, there is conflicting evidence concerning the importance of these risk factors and our findings are supported by other studies. Early intervention studies with long follow up are needed to definitely examine the importance of primary preventive measures against these factors.

The study can not give a definite answer why the prognosis has improved substantially during the last decades in our population. A probable explanation is better metabolic control since the beginning of the 1970s, when the goal of good glycaemic control was emphasized from onset of diabetes. The existence of a diabetes team offered both medical and psychosocial support and encouraged the patients and their parents to take own responsibility for the therapy. Unfortunately there was no possibility to objectively measure the metabolic control before the end of the 1970s and we can just suspect that the metabolic control was worse in the 1960s.

Clinical picture at diagnosis

The clinical characteristics at onset of diabetes were surprisingly constant in our population the last 25 years. The incidence doubled during the time period, but we could not confirm a shift to younger age groups reported from other parts of Sweden¹¹. There seems to be a wide geographical variation of presentation among countries in Europe with association to the background incidence rate of diabetes²⁹². However, the increased incidence in our population seems not to have increased the medical awareness in the general population or among health staff, since the prevalence of DKA, the duration of symptoms prior to diagnosis and HbA_{1c} at diagnosis were unchanged in our population the last decades. The prevalence of DKA was

16%, which is a rather low figure compared to other countries, wherefrom prevalence varying between 20% and nearly 70 % is reported^{220, 292-294}.

C-peptide secretion and partial remission

It was difficult to find a clear secular trend concerning C-peptide secretion. Serum C-peptide values at diabetes diagnosis were higher for the three latest cohorts and fasting, but not stimulated, C-peptide was higher for the cohort 1996 – 2000. In contrast, this cohort had lower values after 1.5 years and longer duration of diabetes. We can not exclude that these values are false low because of too long storage before analysis. A cautious interpretation is therefore, that the pattern of C-peptide secretion was largely unchanged during the late decades. The same was valid for the prevalence of C-peptide after different diabetes duration without clear discrepancy between cohorts. After 1 year more than 90 % of the patients had detectable fasting C-peptide, decreasing to 33% after 5 years of diabetes duration. This is higher than usually reported²²⁴⁻²²⁶. As in other studies we found a correlation between persistent C-peptide secretion and lower HbA_{1c}^{218, 226, 241, 242}. However, fasting C-peptide had to be above 0.1 nmol/l to show a significant association with metabolic control. The causal relationship between C-peptide secretion and metabolic control can not be answered in this type of observational study. The rather good mean HbA_{1c} of 6.3 % (DCCT corrected value 7.4 %) the first 3 years, which is not so far from the intensively treated group (HbA_{1c} 7.2 %) in the DCCT study²⁴², could possibly explain the high persistence of C-peptide secretion in our population.

We defined partial diabetes remission as the period with insulin dose < 0.5 U/kg/day and found an unchanged prevalence except for the cohort with onset of diabetes 1996 – 2000, which had a lower prevalence but unchanged length of the remission period. That does not necessarily mean a real decrease. The insulin dose during the first years after diabetes diagnosis was higher in the cohort 1996 – 2000, probably because of a conscious effort to, when necessary, elevate the insulin dose to achieve the best possible metabolic control and not accept HbA_{1c} values outside the normal range. This will lead to a decreased rate of partial remission with our definition. Several studies have demonstrated a positive correlation between prevalence of partial remission and duration and magnitude of C-peptide secretion^{218, 222, 226, 295-297}. The probably unchanged remission period is in concordance with the unchanged pattern of C-peptide secretion in our population the last decades.

The insulin regimens changed dramatically during the period, which did not apparently affect the remission period and C-peptide secretion. The cohort 1981 – 85 with conventional insulin regimen achieved the same metabolic control during the first years as the following cohorts with more intensive insulin therapy, which shows that factors other than the number of insulin injections is of importance. The insulin regimen per se seems not to influence metabolic control and beta cell function during the first years after diagnosis, which is supported by some small controlled studies^{236-240, 298}.

Conclusions

- ❖ The cumulative proportion of severe retinopathy and overt nephropathy after 30 years of diabetes duration has declined over the last decades.
- ❖ The results were possible to achieve in an unselected population with modern diabetes care.
- ❖ Diabetic nephropathy in this population has probably been prevented and not just postponed.
- ❖ Longer follow up is necessary before we can draw conclusions about whether it is possible to prevent even severe retinopathy.
- ❖ Good glycaemic control was the most important factor to avoid microvascular complications.
- ❖ The risk of overt nephropathy increased substantially when HbA_{1c} exceeded 8.5%, while the risk of severe retinopathy already increased when HbA_{1c} was higher than 7.5%.

- ❖ Intensive insulin regimens from onset of diabetes and fairly good metabolic was not sufficient to entirely escape early microvascular complications after mean 13 years of diabetes duration. The prevalence of subclinical neuropathy was high. The prevalence of background retinopathy was also relatively high, even if it was lower than usually reported, but the prevalence of microalbuminuria was low.
- ❖ Subclinical neuropathy was not a significant risk marker for other complications, which speaks in favour of different pathogenetic mechanisms.

- ❖ The clinical picture at onset of diabetes was unchanged during the last 25 years.
- ❖ Despite intravenous insulin at diagnosis and multiple insulin injections from onset of diabetes, prevalence and duration of partial diabetes remission were unchanged.
- ❖ Magnitude and duration of C-peptide secretion were largely constant during the last decades.
- ❖ Longer persistence of C-peptide secretion during the first five years was not of importance to prevent diabetic retinopathy or nephropathy.

Future research

- ❖ Longer follow up of this population to definite answer the question if we can prevent and not just postpone severe retinopathy.
- ❖ Good long term metabolic control is crucial for prevention of long term complications. We lack studies concerning the long term effect of worse metabolic control during shorter time periods, for example puberty. This is of great interest in clinical practice.
- ❖ Long follow up studies to investigate the prognostic significance of early signs of subclinical neuropathy.
- ❖ Randomized controlled studies to analyse the importance of early treatment of hypertension, lipoprotein disturbances and microalbuminuria in adolescents.
- ❖ Randomized controlled studies of substitution therapy with C-peptide in physiological amounts with long follow up, to determine an eventual beneficial effect on microvascular complications.

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