CAUSE-SPECIFIC MORTALITY AND SURVIVAL AFTER MAJOR COMPLICATIONS IN TYPE 1 DIABETES

DOCTORAL THESIS

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Abbreviations:

EDC	Pittsburgh Epidemiology of Diabetes Complications Study
DERI	Diabetes Epidemiology Research International Study
T1D	Type 1 Diabetes
CAD	Coronary Artery Disease
MI	Myocardial Infarction
ECG	Electrocardiogram
RV	Revascularization Therapy
PTCA	Percutaneous Transluminary Coronary Angioplasty
CABG	Coronary Artery Bypass Grafting
ON	Overt Nephropathy
MA	Microalbuminuria
AER	Albumin Excretion Rate
ESRD	End-stage Renal Disease
RRT	Renal Replacement Therapy
PAI-1	Plasminogen Activator Inhibitor-1
tPA-PAI-1	Tissue Plasminogen Activator-PAI-1 Complex
HbA1	Hemoglobin A1
HbA1c	Hemoglobin A1c
SMR	Standardized Mortality Ratio
CI (95%)	Confidence Intervals (95%)
SD	Standard Deviation
OR	Odds Ratio
HR	Hazard Ratio
RR	Relative Risk

Abstract

Despite the major increase in life expectancy and the development of new technology in diabetes health care, individuals who develop Type 1 diabetes (T1D) remain at substantially greater risk of early mortality. The acute and chronic complications of diabetes might markedly contribute to this. There are only few studies that have accurately quantified the mortality risk associated to complications of T1D. Little is known about the underlying cause of this mortality excess, gender and national/ethnic differences and the temporal changes in mortality by different T1D complications. Once complication developed, survival pattern after preventive strategies becomes important in order to find better approaches to prolong lifetime of those living with T1D.

The current PhD thesis is designed to evaluate the cause specific mortality pattern of T1D through the presence of major diabetes complications (coronary artery disease and renal disease), and to examine demographic factors and causes influencing survival, also after their invasive treatment.

Persons with T1D in the Pittsburgh Metropolitan Area, in Allegheny County, Pennsylvania, USA, in Havana, Cuba and in Pecs, Hungary were identified primarily from population based incidence registries of Type 1 diabetics. Participants diagnosed with T1D between 1965 and 1979, at age <17 years were enrolled into the mortality study. The underlying and secondary causes of death were determined by a Mortality Committee using standardized survey and protocol. Complication data were collected from the Pittsburgh Epidemiology of Diabetes Complication (EDC) Study, a large prospective cohort of childhood-onset (<17 yrs) T1D diagnosed 1950-1979, and from the albuminuria screening program of T1D children (<18 yrs), a hospital-based dataset at the University of Pecs, Departement of Pediatrics.

African Americans (particularly females) and Hispanics had a greatly increased risk of death compared to Caucasians which was attributable to acute complications in African Americans while to renal disease in the case of Hispanics. Males were more likely to die from non-diabetes related causes than females. There was a temporal decline in the 20-year mortality rates in both racial groups across the cohorts diagnosed in 1965-69, 1970-74 and 1975-79. The 5-year prevalence of microalbuminuria was found to be significantly lower among patients with early childhood (0-4 years) onset T1D compared to those in higher age groups (5-9, 10-17 yrs) by diabetes onset. The incidence of overt nephropathy and end-stage renal disease (ESRD) has declined, probably reflecting the better glycemic and blood pressure control available since the early 1980s. Revascularization in coronary artery disease and renal replacement therapy in ESRD can prolong survival after major complications developed.

Magyar nyelvű összefoglalás

Az utóbbi években növekvő incidenciát mutató gyermekkorban diagnosztizált 1-es típusú diabétesz egyre elterjedtebb globális megbetegedés, ami a kora-felnőttkori halálozások nagy részéért felelőssé tehető. A diabétesz akut és krónikus (mikro- illetve makrovaszkuláris) szövődményei jelentősen hozzájárulhatnak a rossz halálozási mutatókhoz. Kevés adatot találunk viszont a magas mortalitási mutatókhoz vezető elsődleges okokról az esetleges nemi illetve származás (rassz) beli különbségekről valamint az utóbbi évtizedek tendenciáiról. A szövődmények kialakulását követően az egyes invazív eljárásokat követő túlélési adatok elemzése segíthet a megfelelő preventív stratégiák kiválasztásában.

Jelen PhD dolgozat megírása során célul tűztem ki az 1-es típusú diabétesz egyes szövődményeihez (kiemelten kezelve a koszorúér betegséget illetve a nefropátiát) köthető halálozási adatok elemzését valamint egyes demográfiai és egyéb tényezők túlélésben (invazív kezelést követően is) játszott szerepének vizsgálatát.

A vizsgálatban Pittsburgh város és vonzáskörzete, Allegheny-megye (Pennsylvania, USA), Havanna (Kuba) illetve Pécs 1-es típusú diabéteszes betegeinek adatát elemeztük. A mortalitás vizsgálatához 1965 és 1979 között, gyermekkorban (<17 év) diagnosztizált és inzulinra állitott betegek követési adatait használtuk fel. A halál közvetlen okát, az ahhoz vezető tényzőket valamint a diabéteszes szövődmények szerepét a rendelkezésre álló orvosi dokumentáció alapján, az e célra kidolgozott standardizált protokoll szerint egy szakértői bizottság állapította meg a végső feldolgozás számára. A szövődmények elemzéséhez a Pittsburgh Epidemiology of Diabetes Complications (EDC) vizsgálat (diagnózis: 1950-1979, <17 év) valamint a Pécsi Tudományegyetem Gyermekklinikáján indított albuminuria szűrés (diagnózis <18 év) adatait használtuk fel.

Az afro-amerikaiak (különösen a nők) valamint a hispán származásúak (Kuba) összmortalitása az amerikai fehér betegek adataival összehasonlítva jóval magasabb értéket mutatott. Ezen különbségek a feketék esetében főként a akut szövődmények, míg a kubaiak esetében a veseszövődmények magas előfordulásának tulajdoníthatók. A nemi összehasonlításban a férfiak között a cukorbetegséghez nem köthető halálokok (pl. öngyilkosság, baleset, rákos megbetegedés) előfordulása magasabb volt. Csökkenő tendenciát figyeltünk meg a 20 éves halálozási adatokban a diabétesz diagnózisának időpontja (1965-69, 1970-74, 1975-79) szerint. A szövődmények elemzésekor a kisgyermekkorban (0-4 év) diagnosztizált cukorbetegek csoportjában a microalbuminuria 5 éves prevalenciája szignifikánsan alacsonyabb volt a későbbi életkorban (5-9, 10-17 év) diagnosztizáltak prevalencia értékeihez képest. A manifeszt nefropátia és a végstádiumú vesebetegség incidenciája az utóbbi évtizedekben látványosan csökkent, feltehetően az 1980-as évektől megfigyelhető folyamatosan javuló glikémiás és vérnyomás kontrollnak köszönhetően. A koszorúérbetegséget követő revaszkularizáció valamint a súlyos vesebetegség esetében használt vesepótló kezelések az adatok alapján megteremthetik a hosszabb túlélés lehetőségét a szövődményektől szenvedő 1-es típusú diabéteszes betegek számára.

1. Introduction

Type 1 diabetes (T1D) is one of the most common serious chronic diseases of childhood. The majority of cases are classified as autoimmune diabetes, which usually is due to destruction of the B-cells of the Langerhans islets, however, non-autoimmune forms (e.g. maturity-onset diabetes of youth – MODY) have been recognized more often in the recent years.

Epidemiology of Type 1 diabetes

Among children, Type 1 diabetes is usually the most prevalent form of the disease. This disorder occurs with an annual incidence of 17 to 18 cases per 100,000 persons younger than 19 years. The prevalence rate is increasing because the incidence is rising and mortality rates are decreasing. One of the more intriguing aspects of the studies in the field of epidemiology of Type 1 diabetes is the remarkable variation in prevalence and incidence in various parts of the world. Incidence figures are lowest in Asia; Japan, Korea, and China report approximately one case per 100,000 people each year. Rates are highest in the Scandinavian countries, especially Finland, and in Sardinia, with incidence rates approaching 40 cases per 100,000 children and adolescents per year. The explanation for these geographic differences is still unclear. Although the frequency of genetic susceptibility haplotypes probably plays a major role among the different races, other populations have very similar gene frequencies. Thus, geographic variations and the rising incidence of T1D around the world point to the role of yet-to-be-determined environmental factors in precipitating insulin deficiency.

Approximately 5-15% of patients with known diabetes have Type 1 diabetes. A large proportion of these patients (probably >60%), acquire the disease before the age of 20, recognition is increasing that the disease may present in adulthood. In childhood, the mean age of onset is ~8 years. A peak is seen in adolescence, which occurs somewhat earlier in girls than in boys. A rise in incidence has been reported among children younger than 5 years. No significant difference is seen in sex distribution of diabetes during childhood.

Future for a child with Type 1 diabetes

In the last one and a half decades, important research advances have been made in patient management. One of the most significant in terms of convenience for the patient with diabetes, particularly the school-age child and adolescent, is the availability of modern analogue insulin short-acting and long-acting insulins, the new easy-to-use insulin delivery devices, the smaller

glucose-monitoring meters and continuous glucose sensor systems and, last but not least, the convenient subcutaneous insulin infusion pumps are becoming widely available. Unfortunately, despite the development of new technology in diabetes health care, individuals who develop T1D remain at substantially greater risk of early mortality. Children with T1D appear to be still much more likely to die than comparably aged individuals in the general population. The complications of diabetes markedly contribute to this.

The Diabetes Control and Complication Trial (DCCT) results unequivocally documented that sustained metabolic improvement in diabetes control is associated with highly significant reductions in the frequency, severity, and rate of progression of the serious microvascular complications. But there are only few large, consistent studies that have accurately quantified the mortality risk associated to acute and chronic complications of Type 1 diabetes. Little is known about the underlying causes of this mortality excess and the temporal changes in mortality by different T1D complications.

Epidemiological research is expected reveal more the causes behind the excess mortality and worse survival and might explain the interrelationships between demographic data, early markers, risk factors, complications and mortality patterns.

2. Review of the literature

2.1. Mortality studies of patients with Type 1 diabetes

Before the discovery of insulin by Banting and Best in 1922, the onset of childhood diabetes represented almost certain death, the life expectancy of a child with Type 1 diabetes was in general not more then two years from diagnosis. After insulin has entered into clinical use, life expectancy for newly diagnosed patients with diabetes has dramatically increased (*Borch-Johnsen K 1989*). However, even as insulin became available widely, it was experienced that childhood diabetes was still associated with markedly excessive premature mortality (*Borch-Johnsen K 1989*). Several decades then elapsed before it was documented in the literature that, even with insulin treatment, diabetic patients are still at a significantly increased risk of dying, not only of acute diabetes-related complications but also of late complications such as diabetic nephropathy and macrovascular disease (*Joslin EP 1950, White P 1956, Entmacher PS 1964*).

2.1.1. Types of mortality studies

Diabetes mortality has been studied by making use of routinely collected vital statistic data, sometimes including more detailed analyses of death certificate data and by conducting follow-up studies involving diabetic patients. The majority of published follow-up studies have been hospital-based (e.g. *Entmacher PS 1964, Dorman JS 1984, Borch-Johnsen K 1985*) and some have presented the outcome in diabetics who has applied for life insurance (*Pollack AA 1967, Goodkin G 1975*). However, still only a few studies have been population-based, following all diabetes patients diagnosed in a certain region over a specified period of time (*Joner G 1991*) or all prevalent patients identified in a defined population (*Moss SE 1991*). Ideally, in a prognostic study patients should be followed from the time of diagnosis in order to avoid selection of a survivor population (*Kaplan MH 1973*).

Vital statistics and death certificates are a readily available source of information on diabetes mortality. Death certificates form the basis of information for vital statistic, however, the results of this type of studies are highly influenced by the certification process. Variation in instructions on filling in death certificates and differences in coding procedures can also influence the evaluation (*Jougla E 1992*). It is not unusual for there to be no mention at all of

diabetes on death certificates, although it is more common to mention diabetes if insulin treatment was given. The majority of death for which diabetes is mentioned on the death certificate are coded under other causes of death (*Fuller JH 1983*) which further reduces the value of routine mortality statistics based on a single underlying cause of death.

The *hospital-based studies* often come from centers with a special interest in diabetes research and treatment. The best-known centers where large-scale follow-up studies on Type 1 diabetes patients have been conducted are the Joslin Clinic in Boston, USA, the Steno Memorial Hospital in Denmark and the Children's Hospital in Pittsburgh, USA. The major potential reasons for selection bias in hospital-based study populations are: 1. referral of patients with complications or poor metabolic control; 2. socio-economically biased referral influenced by income or availability of (private) health insurance and; 3. selective referral of patients owning to the special fields of interest at a centre.

A *population-based mortality follow-up* study requires identification of all people with diabetes in a specified population. The study population of the Children's Hospital in Pittsburgh has been extended to cover all patients with Type 1 diabetes diagnosed in Allegheny County between 1965 and 1979 (*LaPorte RE 1981*) and now comprises one of the few large population-based mortality follow-up studies. The original study population of the Children's Hospital was compared with the population-based register and the patients did not differ in basic characteristics. To avoid bias by selective referral, only patients admitted to the Hospital at the time of diagnosis or at latest within a year of diagnosis were included in the mortality follow-up studies (*Dorman JS 1984*).

2.1.2. Mortality studies in the USA

Several population- and hospital-based studies have been reported by researchers in the USA. The population studies concerning both types of diabetes in the Mayo Clinic, Rochester, Minnesota were relatively small and the number of patients with T1D diagnosed before the age of 30 was low (*Johnson DD 1980*).

Results from the Joslin Clinic showed that 10-year survival of patients diagnosed as having diabetes in the 1930s was higher than 90% (*Marks HH 1965*). Publications from the same clinic indicated that the mortality risk for young diabetic patients was between 12 and 14 times higher than that of the general population, and that there was a marked increase in mortality after 35 years of age (*Marks HH 1965*). The study was, however, potentially biased by an over-representation of individuals with diabetic complications (*Deckert T -I 1978*).

Furthermore, no attempt was made in the Joslin studies to determine the representativeness of the population.

Sultz et al. described the mortality experience of 289 T1D patients from Erie County, New York, who were diagnosed between 1946 and 1961 (*Sultz HA 1972*). During the 15-year follow-up period, seven cases had died. The data indicated that 2% of the children in this cohort died during the first 10 years of diabetes. Although the sample size was very small, this study was the first published population-based mortality examination in the USA.

More recently, a follow-up study from the Children's Hospital of Pittsburgh reported that the mortality of subjects diagnosed between 1950 and 1981 was seven times higher than that of the general population as of 1982 (*Dorman JS 1984*). The study also showed that the 10-year mortality decreased from 4.1 to 1.4% during this period. However, these results do not necessarily represent the mortality of the total diabetic population, because the study was hospital-based.

In a larger population-based cohort in Allegheny County, Pennsylvania, with a 19-year minimum and a 34-year maximum follow-up (duration) of diabetes, the mortality of patients with T1D was more than 5 times higher than that of the general population with an overall SMR of 519 (95% CI 440–672) (*Nishimura R 2001*). Mortality, especially after 15 years duration of diabetes declined during the study's last 30 years. SMRs at 20-year duration of diabetes showed a decreasing tendency over the study period, indicating that in US improving prognosis of diabetic children was greater than that of the general population. The prognosis of African-Americans, however, was much worse than that of Caucasians after 25 years of diabetes, although the decline in mortality seemed to be similar in both Caucasians and African-Americans (*Nishimura R 2001*).

2.1.3. Cross-county comparisons in Type 1 diabetes mortality

International comparative studies offer an excellent method for identifying areas with high number of diabetic complication-related deaths; areas where preventive measures could be implemented. Type 1 diabetes still leads to a two- to tenfold excess risk of mortality in developed countries, whereas in developing countries, a large proportion of diabetic patients die within a few years of diagnosis (*WHO 1990*).

The Diabetes Epidemiology Research International (DERI) group conducted an international study comparing the mortality (as of 1 January 1990) of patients diagnosed between 1965 and 1979 in population-based registries from four countries: USA (Allegheny County, PA),

Finland, Japan and Israel. The mortality in Allegheny County was five times higher than that of the general population (*DERI Study Group 1991, 1995*). Despite the fact that the United States currently has one of the most technologically advanced and costly systems of diabetes care in the world, more than 50 percent of the Type 1 diabetes-associated deaths are estimated to be in excess compared to Finland. European reports of population-based studies from Norway (*Joner G 1991*) and the UK (*Warner DP 1998, McNally PG 1995*) suggest that the mortality of children with Type 1 diabetes was two to three times higher than that of the general population. The report from the UK also noted that the standardized mortality ratios (SMRs) decreased from 981 to 238 during the period 1940–1989 (*McNally PG 1995*). The DERI group has recently demonstrated a considerable variation across countries and the possible risk factors for the excess mortality in Japan and the United States in contrast to Israel and Finland (*DERI Study group 1991, Patrick SL 1992*). These geographic differences highlight countries and societies such as Japan and the USA where mortality is excessive and could be reduced.

By employing frequency adjusted diabetes mortality statistics Matsushima et al. were able to extend the geographic comparisons for the four populations to 24 populations (*Matsushima M 1997*). Mortality data for diabetes in the 0–24 year age group were obtained from World Health Organization (WHO) statistics. The mortality rates were adjusted for the frequency of occurrence of Type 1 diabetes by dividing the mortality rates by the Type 1 diabetes incidence rates which were obtained from the WHO DiaMond project. The results demonstrated an almost 10-fold variation in the risk of dying across countries, with young Japanese and Eastern Europeans having by far the highest mortality (*Matsushima M 1997*). The areas having the best outcome associated with Type 1 diabetes were Northern Europe, Central Europe, and Canada. As the Type 1 diabetes incidence rates were measured in the comparable way, they are considered reliable. The results from the DERI mortality study suggest that there is a high congruence across countries concerning mortality of T1D patients before 25 years of age with a similar proportion of patients being reported on death certificates. It is unlikely that a more than 10-fold difference in mortality risk could be attributed to differential completion of death certificates.

The EURODIAB study examining mortality in Europe in the years after diagnosis but before the onset of cardiovascular and renal complications demonstrates that significant excess mortality persists in the years following the diagnosis of Type 1 diabetes in childhood (*Patterson CC 2007*). Most of the excess of deaths were directly attributable to diabetes, and in the majority of such deaths there was mention of diabetic ketoacidosis. Variation between

countries is evident in this excess mortality, but these results do not suggest that it can be explained by a country's incidence rate or gross domestic product. There has been, however, a shortage of data on mortality in T1D in the Eastern European countries.

Population-based data from two Eastern European countries, Estonia and Lithuania was examined by Podar et al. (*Podar T 2000*). Survival of the patients in the Estonian and Lithuanian cohorts was quite similar, as both countries were part of the former Soviet Union and subject to similar health care practices. When compared to the Finnish cohort, people in Finland had better survival than in the two Baltic countries which is hardly explainable by inequalities in access to health services between Finland and the Baltic states. Furthermore, mortality in the general populations of Esonia and Lithuania has been found to be much higher than that in the developed countries bordering the Baltic Sea (*Wedel H 1992*). Compared with the background population, the survival of childhood-onset Type 1 diabetic patients in the two Baltic states is also worse than it is in Sweden and Norway (*Sartor G 1991, Joner G 1991*), where the SMR is 2, and similar to that in a study from Japan (*Nishimura R 1996*). The most frequent cause of death in these two Baltic countries was diabetic ketoacidosis, the main killer of the pre-insulin era which could be potentially prevented. The occurrence of violent death was also high, in this North-East-European region, particularly in Finland.

Mortality data for cohorts of people with T1D from developing countries are lacking. This might be due to the relatively low prevalence of the disease and the inevitable problems in identification of subjects and ascertainment of death (*McLarty DG 1991, Lester FT 1992*). Data from the African continent showed an unfavorable position for the African population when it comes to diabetes-associated mortality. A study from Tanzania, including subjects with both types of diabetes, has shown a 60% survival rate at 5 years of diabetes duration (*McLarty DG* 1991). This effect is probably caused by poor access to health care, missed diagnosis, and the unavailability of insulin in most African countries (*McLarty DG 1991, Lester FT 1992*). In a report from Cuba on a large cohort of people with Type 1 diabetes with prolonged follow-up, diabetic individuals with onset before 15 years of age had a much better prognosis compared to other developing countries. However, the survival of Cuban diabetics was inferior compared with cohorts from developed countries. This was particularly true when diabetes duration was more than 10 years. After 20 years of disease duration, the Cuban cohort had a cumulative survival rate of 84%, considerably less than the 97%, the 95%, and the 94% reported cumulative survival rate at the same years of follow-up for the Finnish, Israeli, and Allegheny County cohorts, respectively (*DERI Study Group 1991*).

2.2. Coronary artery disease in Type 1 diabetes

A high occurrence of, and mortality from coronary artery disease (CAD) in Type 1 diabetes has been documented since the late 1970s (*Deckert T -II 1978, Christlieb AR 1981*). A 1984 registry reported a 10-fold or greater CAD mortality compared with that expected from USA national data (*Dorman JS 1984*). This very high relative risk, partly reflecting the extremely low CAD death rate in the general young adult population, was subsequently confirmed by Joslin investigators (*Krolewski AS Am J Cardiol 1987*), who reported that those with Type 1 diabetes by 55 years of age experienced a sixfold greater cumulative CAD mortality compared with the rate expected using Framingham Study data. The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) also reported a standardized mortality ratio (SMR) from ischemic heart disease of 9.1 (for men) and 13.5 (for women) for those with a diabetes diagnosis before 30 years of age (*Moss SE 1991*).

Many different studies have examined the early stages of CAD with the purpose to find some easily detectable parameter to prevent cardiovascular events. The excess coronary artery calcification (CAC) in Type 1 diabetes seen in studies from Denver (*Dabelea D 2003*) and London (*Colhoun HM 2000*), compared with general nondiabetic control populations, provide further support to the thesis of accelerated atherosclerosis in the coronary arteries. The Pittsburgh EDC study provides some reassurance that CAC reflects atherosclerosis, as there was a strong correlation between CAC and both clinical disease and cardiovascular risk factors (*Olson JC 2000*). A number of angiographic and autopsy studies further suggest more extensive disease in Type 1 diabetic than in nondiabetic cohorts (*Crall FV 1978, Valsania P 1991, Pajunen P 2000*), as does a study from Oslo (*Larsen J 2002*) that demonstrated, using intravascular ultrasound, that all T1D subjects studied had significant coronary intimal thickening. Coincident with changes in intima-media thickening (IMT) are changes in arterial compliance and distensibility. Endothelial dysfunction has been seen in teenage subjects within the first decade of Type 1 diabetes onset (*Jarvisalo MJ 2004, Singh TP 2003*). Thus, these studies confirm that changes in vascular structure and function occur early in the course of Type 1 diabetes

2.2.1. Prognostic factors

Although the increased risk of premature heart disease in Type 1 diabetes has been recognized for some time, the underlying pathogenesis is still poorly understood. The most likely factor, a priori, to account for this increased risk is hyperglycemia. However, despite recent evidence from the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) study that prior intensive glycemic control reduces cardiovascular disease, the epidemiologic association between glycemia and coronary artery disease (CAD) is surprisingly weak.

In the 10-year follow-up report from the EDC study, lipids (both HDL and non-HDL cholesterol), hypertension, smoking, and a marker of inflammation, i.e., total white blood cell count, predicted total CAD events with some variation according to the type of CAD event (e.g., depressive symptomatology predicted angina, whereas nephropathy and a measure of insulin sensitivity [estimated glucose disposal rate, eGDR – including waist-to-hip ratio, hypertension, and HbA1 based on a substudy of 24 subjects who underwent hyperinsulinemic-euglycemic clamp] predicted hard events). Earlier sex-specific analyses (*Lloyd CE 1996*) suggested that nephropathy was a particularly strong CAD risk factor in men, whereas waist-to-hip ratio and hypertension predominated in women. Intriguingly, glycemic control was not a predictor of CAD, either as a baseline predictor (*Orchard TJ 2003*) or using a cumulative glycemic exposure measure (*Forrest KY 2000*).

EURODIAB, with an 8-year follow-up, largely confirmed these findings, although sex-specific results differed, with proteinuria being independently predictive of CAD in both sexes and waist-to-hip ratio in men (*Soedamah-Muthu SS 2004*). Again, HbA1c was not independently associated with either events or left ventricular hypertrophy (*Giunti S 2005*). Case ascertainment varied across studies, with ECG abnormalities responsible for more than half the events in EURODIAB, compared with only 19% in EDC.

The WESDR also confirmed these findings, where a more limited range of standard risk factors supplemented with detailed retinal characteristics also predicted cardiovascular events, although nephropathy appeared to confound these associations and emerged as a stronger predictor (*Klein BEK 2004*). HbA1c similarly showed only a weak association with MI ($p=0.08$) and no association with angina.

Hyperglycemia has in virtually all studies shown only a weak relationship with CAD events. One exception is a small study (*Lehto S 1999*) of older-onset Type 1 diabetic Finnish subjects

without nephropathy. Further exploration of the Pittsburgh EDC and WESDR studies indicate that glycemia does strongly predict peripheral arterial disease (*Olson JC 2002*), amputation (*Moss SE 1999*), and stroke (*Klein BEK 2004*) but only weakly contributes to the development of CAD. There are number of hypotheses explaining this finding, but the exact cause of the less role of glycemia is still unknown.

Beside the relatively well known traditional risk factors described above, there are also specific risk factors heavily studied in Type 1 diabetes. First, nephropathy clearly emerges as a major predictor, as has been recognized for many years (*Jensen T 1987, Krolewski AS 1985*) and repeatedly confirmed (*Tuomilehto J 1998, Torffvit O 2005*). However, even without nephropathy, CAD rates in Type 1 diabetes are still greatly increased (*Krolewski AS 1985*). Another complication often implicated in CAD risk is autonomic neuropathy (*Veglio M 2002*). Numerous mechanisms may account for premature cardiac death in CAD including associations with subclinical but advanced coronary atherosclerosis, abnormalities in coronary vasomotor capacity, changes in systolic and diastolic function, and lastly, life threatening arrhythmia, the threshold for which is lower in the setting of a relative increase in sympathetic tone, a situation commonly seen in diabetic individuals with sympathovagal imbalance.

Over the past decade, atherosclerosis has increasingly been considered, at least partly, an inflammatory disease (*Ross R 1999*). Several newly recognized factors may contribute to this development (*Jenkins AJ 2004, Ross R 1999, Baynes JW 1999*). E.g. the oxidative modification of LDL, and the immune response it produces, may be one of these key factors, as an association between antibodies to oxidized LDL and incident CAD has been reported (*Orchard TJ 1999*). The resulting immune complexes may induce foam cell formation and damage the endothelium (*Lopes-Virella MF 1992*). The adherence of monocytes is also a key step in this process (*Ross R 1999*), and it is of interest that E-selectin shows a strong, independent prediction of heart disease in Type 1 diabetes (*Costacou T 2005*). Other regulators of adhesion molecules include cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-1β (*Bevilacqua MP 1993*). These and other markers of inflammation have not been extensively studied in the development of CAD.

2.2.2. CAD prognosis and revascularization

Coronary artery disease (CAD) is the major complication of both Type 1 and Type 2 diabetes mellitus. Among most white populations, approximately 75% of those with diabetes will die of cardiovascular disease (*Laakso M 1997*). Haffner and colleagues suggested that those with diabetes alone had the same risk of a future heart attack as did those without diabetes who had had a myocardial infarction (MI) (*Haffner SM 1998*). Although other data suggest that people with diabetes alone are not at such high risk, there is universal agreement that the coronary risk in those with diabetes is 2–4 times greater than in those without diabetes (*Stamler J 1993*). This finding holds true regardless of the nationality, ethnic, or age group to which one belongs. In addition to this much greater risk of having an MI, diabetes greatly increases the postinfarction mortality, case-fatality, and 5-year mortality rates (*Miettinen H 1998 and Herlitz J 1988*). Thus, it is vital to undertake all measures that will prevent the occurrence of the first heart attack as well as the recurrence of heart attacks.

Modern coronary revascularization strategies are based on the results of studies conducted in the 1970s and 1980s defining the benefit of coronary artery bypass graft (CABG) surgery compared with medical therapy alone for the treatment of coronary artery disease (CAD) (*Detre KM 1977, European Coronary Surgery Study Group 1982, CASS Investigators 1983*). These early studies demonstrated that the patients most likely to benefit from aggressive revascularization were those with baseline characteristics indicative of a poor prognosis, including severe angina, triple vessel disease, left main CAD (*Takaro T 1982*), left ventricular dysfunction, and manifestations of severe ischemia (*Yusuf S 1994*). With the development and increased use of PTCA (balloon angioplasty) in the early 1980s, investigators turned their attention from indications for revascularization to determination of the optimal form of revascularization among patients presenting for a procedure. Studies of the Duke University cardiovascular database (*Jones RH 1996*) identified a gradient of benefit associated with PTCA or CABG that was dependent on, and correlated directly with, the extent and location of coronary stenoses.

The relative advantages and disadvantages of each procedure provided the impetus for several multicenter, randomized trials comparing PTCA with CABG in patients with multivessel disease. Among nearly 5,000 patients with multivessel CAD amenable to either PTCA or bypass surgery, long-term survival and survival free of myocardial infarction (MI) were equivalent with either intervention, either separately or in total. With similar quality-of-life

measures and employment status between groups (*Mark DB 1994*), the major difference found between these revascularization strategies was the increased need for repeat revascularization among patients undergoing a primary strategy of angioplasty. Early postoperative cognitive impairment, recognized as an important sequela of cardiopulmonary bypass (*Kolkka R 1980*), was not prospectively assessed in these early studies, although long-term differences were not seen in a small subset of patients randomized between surgical and percutaneous revascularization (*Hlatky MA 1997*). Given the overall results of these studies, PTCA was thought to be a reasonable initial strategy in selected patients with multivessel CAD. However, the publication of the largest of these trials, the Bypass Angioplasty Revascularization Investigation (BARI) (*The BARI 1996*), cast doubt on the generalizability of these findings to all patients meeting criteria in these studies, particularly patients with a history of treated diabetes mellitus.

A particularly poor outcome after an MI in diabetes has led to the search for the optimal way to treat people with diabetes who have CAD. The original BARI trial examined the type of coronary intervention, percutaneous coronary intervention (PTCA) or coronary artery bypass graft surgery (CABG) that may be optimal for those with CAD. In 1996, the BARI investigators found that an initial strategy of CABG, as compared with PTCA, significantly improved five-year survival among patients with medically treated diabetes (*The BARI 1997*). A later analysis found that in those with diabetes, coronary artery bypass grafting reduced the death rate in those who had an MI and also, but less so, in those who did not have an MI (*Detre KM 2000*). This analysis identified two major ways in which CABG protected patients with diabetes. First, there was a strong protective effect of CABG with respect to survival for the relatively few patients with diabetes who had a spontaneous Q-wave myocardial infarction during follow-up. Second, CABG moderately reduced mortality among the majority of patients with diabetes who remained free of myocardial infarction during follow-up. Thus, the type of revascularization (CABG) and the presence of spontaneous Q-wave myocardial infarction interacted with one another in a manner that resulted in greater protection in patients with diabetes. Among the patients without diabetes the protective effect of CABG was substantially less (and was not significant), regardless of whether the patients had a myocardial infarction (*Detre KM 2000*). However, it left open the question of whether those with diabetes whose coronary disease can be managed medically would have a better outcome with initial medical or revascularization treatment.

The revascularization arm of the BARI 2D investigation determined that an early invasive revascularization procedure, combined with aggressive and comprehensive medical

management, reduced mortality (*The BARI 2007*). There was no significant long-term disadvantage regarding mortality or myocardial infarction associated with an initial strategy of PTCA compared with CABG. Among patients with treated diabetes, CABG conferred long-term survival benefit, whereas the 2 initial strategies were equivalent regarding survival for patients without diabetes (*The BARI 2007*).

2.3. Nephropathy in Type 1 diabetes

Diabetic nephropathy remains the major cause of end-stage renal failure in the Western world. It has basically three stages depending on the patient's albumin excretion rate (AER) which has been widely used for diagnosis. The early stage of nephropathy is microalbuminuria (AER: 20-200 μg/min) while overt nephropathy diagnosed with AER>200 μg/min. Renal failure with proteinuria is the end stage of nephropathy when renal function can be also seriously damaged and some form of renal replacement therapy is usually needed.
Although albumin excretion rate (AER) (*Mogensen CE 1984*) and glycaemic control (*Warram JH 2000*) are important risk factors, and hyperlipidaemia (*Fried LF 2001*) is thought to enhance kidney disease progression, these factors alone do not adequately explain the inter-individual variation in risk of developing nephropathy.

2.3.1. Early microalbuminuria

Microalbuminuria (MA) is an important early marker of risk for diabetic renal disease. In adults with Type 1 diabetes, between 20 and 80% of subjects with MA are expected to progress to severe renal disease (*Viberti GC 1982, Almdal T 1994, Watts GF 1991*). MA is also associated with increased risk for cardiovascular disease and early mortality (*Rossing P 1996, Borch Johnsen K 1985*). The development of MA is linked to duration of diabetes in adults (9–11), and it is therefore less common in children and adolescents, for whom prevalence rates vary between 4 and 21% (*Quattrin T 1995*). In children, as in adults, the development of MA appears to be related to glycemic control and duration of diabetes (*Davies AG 1985, Quattrin T 1995*). However, estimates of the prevalence of MA during childhood are confounded by the effects of puberty, as indicated by the documentation in several studies that MA is rare before puberty, even in individuals with diabetes of long duration (*Norgaard K 1989, Rudberg S 1993*). Furthermore, most studies of MA in children have been cross-sectional, with only a few being longitudinal (*Almdal T 1994, Rudberg S 1993*). When longitudinal studies have been undertaken, they have been clinic- rather than population-based, and the sample sizes have been relatively small (*Barkai L 1998, Jones CA 1998*). Determination of the prevalence of MA in relation to age, sex, duration of diabetes, glycemic control, and puberty and of the predictive value of MA in childhood was purposed in a large prospective population-based study (*Schultz CJ 1999*). These data confirm that the

cumulative probability of developing MA during childhood is related to sex, HbA1c, duration of diabetes, and pubertal status. Differences in pubertal status have a profound effect on the prevalence of MA, and that before puberty, there is a latent period followed by a more rapid development of MA after pubertal onset.

2.3.2. Overt nephropathy

Overt diabetic nephropathy (ON) carries a high risk of mortality in Type 1 diabetes not only in its own right (*DERI group 1990*), but also as a risk factor for coronary artery disease (CAD) and considered to be as an important stage before end-stage renal failure.

Incidence rate of overt nephropathy increases linearly in the EDC study reaching 2% by 28 years of diabetes duration which is higher than those reported from Linkoping (*Nordwall M 2004*), but very consistent with recent Steno data (*Hovind P 2003*). A major shift in the incidence pattern of ON has likely occurred compared with the earlier Steno data (*Kofoed-Enevoldsen A 1987*). With loss of the "16-year peak," it is important to note that incidence is only being delayed until the later years. After 20 years' duration, the incidence is in fact higher in the more recent EDC data than the earlier Steno data. The explanation for the much lower Linkoping rates is undetermined but may relate to methodological differences in follow-up, as well as reflecting a truly lower level of complications and better glycemic control (*Nordwall M 2004*).

The nature of ON's association with CAD is intriguing, for despite the strong association seen in the initial reports from the Steno Clinic (*Borch-Johnsen K, 1987*), recent observations from both the United States (*Forrest KY-Z 2000*) and Europe (*Koivisto VA 1996*) suggest the association is largely, if not totally, explained by disturbed vascular disease risk factors, notably blood pressure, lipids/lipoproteins, inflammatory markers, and smoking. ON also appears to be a stronger risk factor for CAD in men than in women with Type 1 diabetes (*Forrest KY-Z 2000*).

Several studies provide support for an insulin resistance-renal link in Type 1 diabetes. Yip et al. have reported that T1D subjects with MA had significantly lower glucose disposal during euglycemic-hyperinsulinemic clamp studies than did matched controls, even after accounting for blood pressure and body mass index (*Yip J 1993*), while De Cosmo et al. drew attention to the high prevalence of CAD risk factors in parents of Type 1 diabetes subjects with albuminuria (*DeCosmo S 1997*). These authors also recently suggested that the rate of progression of ON may be predicted, in Type 1 diabetes, by a variant of an inhibitor of insulin

signaling PC1 glycoprotein (*DeCosmo S 2000*). A further set of data also suggest that the angiotensin-converting enzyme insertion/deletion (ACE I/D) polymorphism is associated with predisposition to both ON in Type 1 diabetes and to insulin resistance (*Panahloo A 1995*). The evidence of whether ACE inhibition improves insulin sensitivity and glucose tolerance is also controversial with some reports showing no effect (*Jandrain B 1992*), while others suggest an improvement in insulin sensitivity and glucose levels with use of ACE inhibitors (*Pollare T 1989*).

The major findings in the 10-year follow-up data of the Pittsburgh Epidemiology of Diabetes Complications Study, a well-characterized population-based cohort of Type 1 diabetes patients, are the clear demonstration that insulin sensitivity (measured by an estimation – eGDR) is the predominant predictor of ON along with white blood cell count and that blood pressure (beyond any association with eGDR) and lipids appear to only predict in the short term (*Orchard TJ Diabetes 2002*). This suggests that the latter mainly act as late-stage accelerators or precipitators rather than underlying etiologic factors. In addition, this study also presents a novel way to explore genetic susceptibility (*Orchard TJ Kid Int 2002*). Urinary albuminuria (AER) was found to be a strong independent predictor of overt nephropathy in both univariate and multivariable analyses in the EDC study (*Yishak AA 2006*), in agreement with earlier reports (*Mogensen CE 1984*).

2.3.3. End-stage renal disease and renal replacement therapy

End-stage renal disease is the final stage of nephropathy when renal function is usually seriously impaired. Thus, life-quality and life expectancy becomes very poor in patients making this entity which is one of the most severe complications of Type 1 diabetes.

Hasslacher at al. have reported that the prevalence of proteinuria, 25 years after diagnosis of T1D, was about 40% (*Hasslacher C 1989*). Krolewski et al. showed that about 40-50% of subjects with T1D developed diabetic nephropathy in their life time (*Krolewski AS N Engl J Med 1987*). The same author reported also that approximately 30 % of T1D have ESRD at 40 year after onset. (*Krolewski M 1996*). Klein et al. reported that cumulative incidence of renal insufficiency (serum creatinine >=2.0 mg/dl) and ESRD in a population-based cohort from Wisconsin was 14.4% at 10 year (*Klein R 1999*). In the Pittsburgh Epidemiology of Diabetes Complications study based on children diagnosed with T1D between 1950 and 1980, the cumulative incidence of renal failure at 25 years has declined from 21% to 9% for those diagnosed in the 1950's to the 70's, respectively. (*Orchard TJ Diabetes 2002*).

Because diabetes is the leading cause of end-stage renal disease (ESRD) (*Centers for Disease Control and Prevention 2005*) and testing for proteinuria is widely recommended for patients with diabetes, the sensitivity of albuminuria in identifying persons at risk of progressive loss of kidney function is utmost important in all types of diabetes. A report from the UK Prospective Diabetes Study (UKPDS) concerning patients with newly diagnosed Type 2 diabetes indicated that 51% of patients who developed renal impairment did not have preceding albuminuria (*Retnakaran R 2006*). Similar findings were observed in the DCCT/Epidemiology of Diabetes Interventions (EDIC) Study, in which a substantial proportion (44%) of individuals with Type 1 diabetes and eGFR less than 60 mL/min/1.73 m^2 presented with normal AER (*Molitch ME 2006*). In the EDC study, contrary to previous reports, development of ESRD without albuminuria may occur rarely in patients with Type 1 diabetes (*Costacou 2007*). Individuals with this manifestation in this cohort used greater insulin doses per body weight despite insulin sensitivity (according to the eGDR) and HbA1c levels similar to persons with albuminuria when seen in our study.

After patients had ESRD, mean survival after the introduction of renal replacement therapy (RRT) is extremely poor (*Matsushima M 1995, McCrary RF 1981*). However, a declining mortality has been reported in other studies after the introduction of kidney or pancreas-kidney transplantation (*Becker BN 2000, Wolfe RA 1999*). Little information is available,

however, regarding the exact incidence rates of ESRD and its related mortality in childhood-onset Type 1 diabetes from population-based studies.

3. Objectives

The aims of the present studies were to determine and analyze cause-specific mortality and complication related survival patterns in patients with T1D, specifically:

1. to determine mortality rates related to acute and chronic diabetes complications and to analyze to role of CAD and nephropathy in the underlying causes of death in the Pittsburgh Metropolitan Area (Allegheny County, Pennsylvania, USA)

2. to compare two T1D populations living under considerably different social and health care circumstances in terms of cause-specific mortality patterns (Allegheny County registry vs Havana cohort)

3. to estimate the relative mortality of patients with T1D as compared to the general population in relation to age and duration of diabetes, and to analyze the effects of age at diagnosis, year of diagnosis in both populations

4. to examine racial (Caucasian, African-American, Hispanic) and gender differences in cause-specific mortality in both cohorts

5. to analyze the incidence and predictors of CAD and different stages of nephropathy (MA, ON, ESRD), as well as the survival after these complications, and their invasive treatment (coronary revascularization (PTCA, CABG) and renal replacement therapy (dialysis, kidney transplant)) in Allegheny County.

4. Patients and methods

4.1 The Diabetes Epidemiology Research International (DERI) Study –

Epidemiology of Type 1 Diabetes Mortality in Four Countries

The DERI study is one of the largest population based prospective cohort study of T1D mortality which was initiated in 1986 to test the hypothesis of cross-population differences in diabetes prognosis. The study has evaluated mortality in comparable population-based cohorts from four distinctly different countries (USA, Finland, Japan, Israel). These areas were selected because they were racially and culturally diverse, had advanced diabetes-care systems, had major cross-population differences in the risk of developing childhood onset diabetes and had comparable population-based cohorts. Of particular importance, they were also different patterns in the background mortality.

4.1.1. Identification of comparable cohorts

This study evaluated four population-based cohorts of T1D cases from Japan, Israel, Allegheny County, Pennsylvania, and Finland. In order to make cross-country comparisons, the Allegheny County, Pennsylvania cohort was taken to be representative of the United States.

Inclusion in the study was based on the following criteria: 1. subjects were diagnosed with diabetes before 18 years of age between January 1, 1965 and December 31, 1979; 2. placed on insulin at diagnosis; 3. residing in the geographic areas listed above.

Persons were excluded if they developed diabetes from a secondary cause, i.e., diabetes associated with cystic fibrosis or Down's syndrome and steroid-induced diabetes.

Eligible individuals in Israel, Allegheny County, and Finland were identified from incidence cohorts. In contrast, the individuals from Japan were identified from two prevalence cohorts, and follow-up was identified as of the date each prevalence cohort was identified rather than at the date of diagnosis. The first cohort consists of individuals who were diagnosed between January 1, 1965, and December 31, 1969, and were alive as of January 1, 1970. The second cohort consists of those who were diagnosed between January 1, 1970, and December 31, 1979, and were alive as of January 1, 1980. In order to make the three incidence cohorts comparable to the prevalence cohorts in Japan, this "alive as of' criterion was applied to the

three incidence cohorts. Consequently, individuals who fulfilled the criteria of the first cohort and died before January 1, 1970, and those who fulfilled the criteria of the second cohort and died before January 1, 1980, were excluded.

The cohort of Allegheny County, Pennsylvania, USA:

Allegheny County is located in southwestern Pennsylvania, which hosted a population of approximately 1.6 million in 1970 and 1.3 million in 2000 with Caucasians accounting for more than 80% of the population. The city of Pittsburgh is the seat of the county as well as the western Pennsylvanian region.

Persons with T1D in Allegheny County were identified from an incidence registry developed through the review of hospital records and validated by contact with pediatricians in the community. Ascertainment of the cases was accomplished by retrospective review of hospital records. For this registry, the degree of ascertainment is well over 95 percent. To be included in the registry, the patient had to be on insulin therapy at hospital discharge. Allegheny County has one large children's hospital (Children's Hospital of Pittsburgh) and 25 general hospitals that possibly would see young diabetics. All but one of the hospitals were surveyed (permission was not obtained for one small hospital). Few cases were likely to have been missed by this omission because of its size. In addition, physician records from two large private practices were also reviewed. Monthly listings of discharges as compiled by the Hospital Utilization Project were carefully inspected in each hospital and records were requested for all discharges where diabetes was the primary diagnosis and the patient was less than 20 years old. For each case, demographic information, date of diagnosis, referring and attending physicians, history of onset of symptoms and family history of diabetes were recorded. Subjects identified only from readmission records were included only if verification of residence was obtained from municipal telephone books for the year of diagnosis. Hospital records at the time of diagnosis were obtained for over 90% of the cases. 50% of T1D cases were diagnosed at the Children's Hospital. Another 20% diagnosed at other hospitals were seen later in Children's Hospital's diabetes programs. Thus, although there were 26 hospitals in the county, over 70% of the diabetics were eventually seen at Children's Hospital and could be identified through review of that hospital's records. To check the completeness of ascertainment, all pediatricians (approx. 150) in Allegheny County were requested to identify children under their care who met the criteria for inclusion in the registry. This survey revealed that all children fulfilling the criteria were seen in a hospital. These results gave

confidence that an accurate hospital record review provides virtually complete ascertainment of cases developing in the county.
From the Allegheny County registry, 1,075 eligible cases were identified as of January 1, 1980.

4.1.2 Procedures for mortality and complications

In order to follow up cases, permission was first obtained from the hospital at diagnosis to contact the attending or referral physician for each eligible case. Upon approval, permission was then obtained from the attending/referring physician to approach patients, who were contacted by letter, and, where needed, by phone at initial follow-up conducted in 1985. To ascertain living status, the same follow-up procedure was done as of 1990. As of January 1, 1999, living status was updated with the addition of a complication survey with nine new questions concerning renal, retinal, cardiovascular and peripheral artery status (*Appendix 1*.)
This questionnaire included yes/no answer possibilities to the complication questions with asking also about the date of complications' onset. Specifically, the diagnosis of coronary artery disease (CAD) was based on history of myocardial infraction and/or revascularization therapy as well as CAD related death confirmed by death certificate. Similarly, onset of end-stage renal disease (ESRD) was defined as the introduction of renal replacement therapy (dialysis or transplantation). Transplant recipients also were asked if they underwent kidney transplantation or pancreatic-kidney transplantation.
Re-contact was first made by a letter with a questionnaire form to sign giving consent. If the subjects did not respond to this questionnaire, they were contacted by phone. Names of patients for whom the investigators could not ascertain their living status by mail or phone contact were submitted to a National Death Index (NDI) search to see if these patients are deceased or not. For patients whose deaths were confirmed by NDI, death record follow back investigation was conducted to obtain the permission from next of kin to obtain the medical records surrounding death for in hospital deaths and coroner's and/or autopsy report for out of hospital deaths.

4.1.3. Validation process of causes of death

For the cases identified as having died attempts were made to collect data from several sources for events surrounding the death. First of all the death certificates were obtained. In addition, copies of the hospital records surrounding the death and autopsy records (if available) were obtained in order to establish the cause of death more firmly. A standardized set of information abstracted from the medical records was created by trained personnel, mainly by the co-investigator of the study. This person had a medical degree and had also a good understanding on the field of diabetes treatment and research. The information sheet collected the past medical history and all the details concerning the death. The past medical history focused particularly on kidney and cardiovascular disease, previous admissions for diabetes control, and evidence of potential self-destructive behavior. The second set of information consisted of circumstances surrounding the death and complication status, with particular focus on kidney disease, current method on insulin treatment, and recent cardiovascular events. Details of recent infections were also collected. In the case of accidents and suicides, further relevant information was documented. In the event that a hospital record was not available, similar information was obtained from physician records, medical/legal reports, or, as a last resort, informant interviews. In this case, a study physician (usually the principal investigator or the co-investigator) or nurse asked about the circumstances at death if the next of kin previously agreed to talk. In case if they felt uncomfortable at any point they were free to stop the interview. Autopsy data were also sought for individuals who had been hospitalized and underwent an autopsy. The percentage of subjects autopsied was different in the participant countries, but was about 50% in Allegheny County in contrast with Finland where around 90% of subjects were autopsied. For those who died outside hospital, alternative sources of information were sought. Coroner's/medical examiner's investigations and police reports were obtained, and detailed abstracts were made by trained abstractors. Standardized data were also obtained from autopsy reports. In all cases, details from death certificates were fully abstracted. Standardized forms were used for all data collection and were transmitted to the central coordinating center in Pittsburgh, Pennsylvania.

Mortality classification:

The objectives of the mortality classification, with all available information, were to determine 1. the most likely underlying cause of death, 2. the order of importance of all other contributing causes of death, and 3. the role of diabetes in causing the death.

Given the complex interaction of various potential causes of death, e.g., kidney failure and myocardial infarction, it was decided to have the secondary causes listed in an ordered manner according to the role in causing death to permit standard combinations of causes of death. A set of standardized rules for classification of death was developed during the review process. Whenever a new rule was adopted, it was applied to any previous relevant case. For example, although diabetes is a recognized risk factor, it was decided not to list diabetes as a contributing cause unless it contributed to the death in other ways (e.g. by the development of diabetic ketoacidosis).

The evaluation of the role of diabetes was deemed as being critical to understanding mortality. The following three levels of contribution of diabetes to death were designated: *level 1*, diabetes caused the death (i.e. death would have occurred from a diabetes cause regardless of other conditions that were present); *level 2*, diabetes contributed significantly to the death (i.e. deaths would not have occurred if the patient had not had diabetes, but another potentially lethal condition was present and also contributed); and *level 3*, diabetes contributed marginally to the death (i.e. diabetes was not thought to be essential to explain the death but played a role). To facilitate an understanding of the process leading to death, schematic diagrams were drawn for each death by the reviewers.

The review procedure was handled as follows. A Mortality Classification Committee (MCC) supervised the validation in each case. The MCC consisted of four physician members, one from each country and an independent chairman. Each member reviewed all collected data before each mortality review meeting and completed a review form that addressed the three designated objectives. For each death the MCC member wrote a brief summary in English of the circumstances leading to death without making any judgment about their interpretation of the underlying cause. In this manner, a standardized set of information was coherently collated for each committee member, although members were free to interpret the clinical data recorded in the forms independent of the summary. The specific methods of operation was fully described and made available for all committee members. All deaths were presented by the member from the respective country and reviewed by the committee. Discussion then took place as to the key points underlying cause of death, order of importance of contributing

causes, and the role of diabetes. If after discussion, all four members did not come to a unanimous agreement, the independent chairman had a casting vote. This, however, rarely occurred. This procedure allowed that the patterns of death across countries became directly comparable. Though the committee members could not be blinded to the deceased patient's country of origin, the standardized approach and full discussion ensured, so far as possible, to immunize them from any potential biases, that may have arisen from knowing the county of origin.

4.1.4. Ethical consideration

The whole study procedure was approved by the Institutional Review Board of the University of Pittsburgh. All information gathered in this study was protected against release to unauthorized people. Any information, which carried personal identifying material, was kept in locked files. Identity of the patients has not been revealed in any description or publication of this research.

Contacting next of kin was initially conducted by mail. If the next of kin did not want to sign the release form, they could refuse it by checking the box at " I do not wish to sign" on the contact letter. If the next of kin did not respond to the letter, an experienced physician or nurse called them and asked them if they received the letter from us and if they had agreed to sign a medical release. When contacted by phone, a trained physician (usually the investigator/reviewer) or nurse asked about the circumstances at death if the next of kin agreed to talk. They were told that if they felt uncomfortable at any point they were free to stop the interview and the investigators recontacted them only at their invitation.

4.2. The Pittsburgh Epidemiology of Diabetes Complications (EDC) Study

The EDC study is a representative (*Wagener DK 1982*) originally 10-year prospective study examining the prevalence, incidence, interrelationships and risk factors of the various complications of Type 1 diabetes, now entering its 20th year of follow-up. The EDC baseline examinations were conducted between 1986 and 1988, and participants were examined biennially thereafter. A total of 1,124 patients were eligible for the study, having been diagnosed or seen within 1 year of diagnosis at Children's Hospital of Pittsburgh between 1 January 1950 to 31 May 1980 and living within 100 miles or 2.5 hours from Pittsburgh. All were receiving insulin therapy at the time of initial discharge from the hospital. Of the 1,124 eligible patients, 145 (13%) had died before baseline (1986–1988) and 191 (17%) chose not to participate. A total of 788 (70%) participated, with 130 (16%) providing survey information only. Thus, 658 subjects met eligibility criteria (325 women and 333 men) and participated in the baseline examination in 1986 to 1988 where the mean age was 28 years and diabetes duration was 19 years. Those refused clinic attendance completed a medical history questionnaire thereafter.

4.2.1. Patient selection for the CAD and ON complication study

Four hundred and fifty-four participants (223 women, 231 men, mean age: 32 ± 8 years, mean duration of diabetes of 23 ± 8 years) were selected who attended the third biennial follow-up visit (1990-1992). The incidence data of CAD and ON were collected between the third and sixth visits (1990-1998), while clinical and metabolic risk factors were gathered from the third cycle of examinations in 1990 to 1992. The average follow-up time was six years.

4.2.1.1. Clinical evaluation and metabolic investigation

Clinical evaluation and definition of T1D complications used in the EDC study have been reported in details (*Orchard TJ 1990 I-II*). Body-mass index (BMI) was calculated as body weight divided by height squared. Waist circumference was measured on standing subjects midway between the lowest rib and the iliac crest. Hip circumference was measured over the widest part of the gluteal region and the waist to hip ratio (WHR) was calculated. Blood pressure was measured according to a standardized protocol (Hypertension Detection and

Follow-up Program) (*Borhani NO 1976*). Hypertension (HTN) was diagnosed as use of blood pressure medication or a level of 140/90 mmHg or above. Those who had ever smoked at least 100 cigarettes and still smoked at baseline were considered to be smokers.

Coronary artery disease was defined by a history of myocardial infarction, confirmed by electrocardiographic changes (Minnesota codes 1.1 or 1.2) at time of examination, or review of previous medical records by use of standardized criteria (*Orchard TJ 1985*), angina diagnosed by the clinic physician, ischemic ECG (Minnesota codes 1.3, 4.1, 4.2, 5.1, 5.2, 7.1) or coronary artery stenosis ≥50% by angiogram. Overt nephropathy was defined by renal failure or an albumin excretion rate >200 μg/min in at least two of three timed urine specimens.

Glycosylated hemoglobin (HbA1) was originally determined with saline-incubated blood samples and microcolumn cation-exchange chronomatography (Isolab, Akron, OH). However, from October 1987 the technique was changed to high-performance liquid chromatography (Diamat, Bio-Rad, Hercules, CA) which is relevant from the CAD/ON complication subgroup analysis. Serum cholesterol and triglyceride levels were determined enzymatically. High-density lipoprotein cholesterol was determined by a heparin and manganese procedure. Low density lipoprotein cholesterol was calculated with the Friedewald equation if triglycerides were <4.5 mmol/l. Fibrinogen levels were performed via a biuret colorimetric procedure and a clotting method and creatinine with an autoanalyzer (Ectachem 700xr, Kodak, Rochester, NY). PAI-1 and tPA-PAI-1 concentrations were determined by an ELISA procedure (American Bioproducts Co. Parsippany, NJ, USA). The PAI-1 assay measured total circulating PAI-1 (i.e. free or complexed with tPA). The intra- and interassay coefficients of variance for PAI-1 were 5.9% and 9.4%, respectively, and for tPA-PAI-1 8% and 11%, respectively. In this subgroup analysis, there were 13 and 15 individuals in which PAI-1 and tPA-PAI-1, respectively were not obtained for technical reasons. Beck Depression Inventory was also used to assess psychosocial status (*Beck AT 1988*). Estimated Glucose Disposal Rate (eGDR) was calculated based on equation (eGDR=23.31-12.22(Waist to Hip Ratio)-3.29(Hypertension)-0.57(HbA1)) derived from hyperinsulinemic-euglycemic clamp studies (*Williams D 2000*) to determine insulin resistance in a subgroup (n=24) of the EDC population.

4.2.1.2. Ethical considerations

The EDC protocol was approved by the University of Pittsburgh Institutional Review Board, and all patients gave informed consent.

4.2.1.3. Statistical analysis

For those variables not normally distributed (i.e. PAI-1, tPA-PAI-1, triglycerides, Beck score, white blood cell counts) logarithmic (log10) transformation was performed. Two-sided Student's t-test and the Mann-Whitney U-test were used to examine differences in PAI-1, tPA-PAI-1 and risk factors between incident cases and non-cases. Chi square test was applied to reveal differences in categorical variables. Spearman's rank correlation and partial correlation were used to assess univariate associations between baseline variables.
Cox regression modeling (forward stepwise method; p-value for entry: 0.05, removal: 0.1) was used to examine relationships between T1D complication status and independent variables (e.g. PAI-1, tPA-PAI-1, HbA1, age, triglycerides, hypertension, Beck score, leukocyte count, fibrinogen).

4.3. Combined cohort of DERI+EDC studies

For wider mortality analyses the patient group of the DERI study was extended with EDC individuals based on standardized procedure. Thus subjects involved into the combined cohort were identified from two Type 1 diabetes incidence registries, the Allegheny County, PA Registry and the Children's Hospital of Pittsburgh Registry (*Dorman JS 1984, Orchard 1990 I-II*). Seven hundred and fifty four subjects came from the Allegheny County registry alone (63 of whom were Black), 245 from the Children's Hospital (5 of whom were Black) and a further 262 subjects were members of both cohorts (8 of whom were Black and 1 Asian who for analysis is included as Black), giving a total of 1261 subjects. Allegheny county cases were identified as described by the DERI study (periodic review of hospital records and validated by contact with pediatricians in the community yielding a >95 percent ascertainment rate).

The eligibility criteria for the current combined analysis were: 1. a diagnosis of Type 1 diabetes between January 1, 1965 and December 31, 1979; 2. age <17 years old at diagnosis; 3. receiving insulin therapy at discharge from diagnosis hospital admission; and 4. residence of Allegheny County, Pennsylvania or living within 100 miles from Pittsburgh at diagnosis. Persons were excluded if they had developed diabetes from a secondary cause, e.g. diabetes associated with cystic fibrosis, Down's syndrome, or steroid therapy as described in the DERI methods.

Vital status ascertainment has been described earlier as it follows the procedure seen in the DERI study (*Nishimura R 2001*, *Orchard 1990 I-II*). Briefly, living status as of January 1, 1999, was verified by letter or if needed, telephone contact (Allegheny County Registry cohort) or by attendance at biennial examinations for the Children's Hospital of Pittsburgh subjects participating in the Epidemiology of Diabetes Complication Study (EDC). The living status was determined for 1183 (94 percent) patients of whom 200 have died.

4.3.1. Determination of causes of death

The underlying cause of death was determined by the review of death certificates, hospital records, coroner's records, and autopsy reports using the standardized protocol of the Diabetes Epidemiology Research International Group Mortality Classification Committee

(DERI) [see in section 4.1.2.] and verified by a committee of physicians based on a standardized protocol [see in section 4.1.3.].

For mortality analysis, the underlying cause of death was grouped into acute diabetes complications (deaths from diabetes ketoacidosis, hyperglycemia, hypoglycemia, and unspecified coma); chronic complications (deaths from renal, cardiovascular diseases, infection and other diabetes related problems), and other causes (deaths from non-diabetes related problems, e.g. accident/suicide, cancer and post-surgical complications).

4.3.2. Statistical analyses

Overall, cause-specific mortality rates per 100 000 person years of follow-up and 20-year cause-specific rates by sex and race were determined. To assess the temporal trends of cause-specific mortality, the total cohort was divided into three groups by year of diagnosis (1965–69, 1970–74 and 1974–79). Temporal trends were also assessed by sex and race. The chi-square test for trend was used to assess the trend of mortality according to diagnosis cohort. Chi square or Fisher's exact test was used to determine any difference between categorical variables. Lifetable analyses by the Kaplan–Meier method were also performed, and the log-rank test was used to determine the statistical difference between the hazard curves. We used Cox proportional hazard models to simultaneously estimate hazard ratios (and 95% confidence intervals) for the effects of sex, race, age at diagnosis and year at diagnosis. The 95% confidence intervals were determined based on the Poisson distribution [14]. Statistical analyses were conducted with SPSS software version 12.1 (Statistical Package for the Social Sciences, Chicago, IL, USA). A two-sided *P*-value of less than 0.05 was considered statistically significant.

4.4 The Havana City cohort

The Havana city cohort is created to determine the survival pattern and the underlying cause of death in a cohort of childhood-onset T1D subjects from Havana City Province, Cuba. The mortality evaluation based on this cohort was first report of a study performed on a large cohort of people with T1D followed up for a long time in a developing country (*Collado-Mesa F 1997*).

A Havana city cohort was defined by age at diagnosis of T1D (<15 years of age), place of residence at diabetes diagnosis (Havana City Province), and date of diagnosis (1 January 1965 to 31 December 1980).

Inclusion into the cohort began at the onset of T1D, and the patients were followed until they died, emigrated, or until 31 December 1991. Emigrated subjects were censored at the date of migration. Subjects with unknown status at the end of the study were censored at the date of the last medical consultation.

To estimate the number of subjects in such a cohort, the investigators examined Havana City Province data from the National Registry of T1D subjects with onset before 15 years of age. This Registry has been running since 1979, and in 1990, it was enrolled as a participating center of the World Health Organization (WHO) Multinational Project for Childhood Diabetes (DiaMond Project) (*WHO DiaMond Group 1990*). The average annual number of incident cases between 1979 and 1990 in the province was 30. Thus, the estimated number of T1D subjects during the 15-year period of cohort formation (1965-1980) is 450, assuming no temporal changes in the disease incidence and population demographics.

The cohort was assembled from several sources. Between 1965 and 1980, there was a single health care center (Hospital Dr. Pedro Borras Astorga), where all children were diagnosed with T1D and where all children living in Havana City Province attended. At age 15 years, they were all referred to another center for teenage diabetes (Diabetics Care Centre, National Institute of Endocrinology). In this center, a registry, including all data from the pediatric health care center, was maintained. Those on this registry were included in the cohort.

Some children with T1D may have died before they could be referred to the pediatric health care center. Therefore, examined necropsy records of pathology archives at pediatric hospitals in Havana City were also examined for the study period.

All residents of Cuba have a unique identity number at the Identity Card and Population Registry. This number was used to obtain the subjects' vital status at 31 December 1991. All deaths and migrations in Cuba are registered here.

A single National Public Health System has been running in Cuba for more than 35 years, providing free medical care and covering the entire population. Death certificates are completed by medical doctors for every death in the country. The National System of Public Health Statistics collects and produces data of recognized quality.

Since information on death certificates is imprecise (*DERI Study Group Am J Epidemiol 1991, Karger S In Prognosis of Diabetes in Children 1989)*, the investigators attempted to obtain more detailed information from clinical records and necropsy reports (if performed), using a similar approach to that used by the Diabetes Epidemiology Research International (DERI) Epidemiology Mortality Group (see section 4.1.2.) (*DERI Study Group Diabetes Care 1991)*.

After all deaths in the cohort were identified, attempts were made to obtain copies of the medical records (including the last hospitalization if the subject died in a hospital), autopsy reports, and death certificates. Standardized forms were used for all data collection. Information on cause of death was obtained from death certificates in 90% of deceased subjects, from necropsy reports in 64.3%, and from medical records in 31.4%.

A one-paragraph summary on terminal events was then prepared. The three members of the mortality classification committee independently coded the main cause of death and secondary causes that contributed to that death. Eventually, all the information was evaluated by the committee, which collectively decided the most likely underlying cause of death for every deceased subject.

4.4.1. Combined cohort for comparative analysis

For further examine and compare the mortality in Havana, Cuba and in the Allegheny County, Pennsylvania, USA, a standardized cohort from these two population-based registries was created. Cases diagnosed with T1D between 1965 and 1980 in the Havana cohort and between 1965 and 1979 in the Allegheny County cohort were included. The inclusion criteria for both registries were: diagnosed by a physician as having diabetes, placed on daily insulin injection before the 15th birthday and resident of the registry caption area at the time of first insulin administration.

Cases in Allegheny County cohort were identified from an incidence registry (see section 4.1.1.). The Havana cohort was assembled with cases from various sources. The Havana

registry was validated by comparison to a census of people with diabetes in three health areas of about 30,000 inhabitants each. All the cases meeting the inclusion criteria found in the community had been previously included in the registry. Therefore the registry was considered virtually complete.

For the analysis of causes of deaths both studies followed the procedures described by DERI study (see section 4.1.2.) which included information from death certificates, necropsy or coroner's reports and hospital records. The deaths in the Havana cohort were classified by three well-trained physician-members of an ad hoc committee. Deaths in Allegheny County were classified by physician members of the DERI Mortality Classification Committee (see section 4.1.3.).

Follow-up started with the onset of diabetes in each individual and ended as of January 1st 1991, or with death.

4.4.2. Statistical analyses

The mortality in the general population in Cuba (*Ministerio 1996*) and the US (by race) (*US Census 2000, US Department 1996*) in 1980 were used as background mortality in the calculation of SMR. Mortality rates and SMR were adjusted by age and sex using the world population as standard (*M. Segi 1963*).

Life-table analyses were used to examine the mortality rates in both populations by duration of diabetes. To determine age-specific mortality rates, the number of deaths that occurred within each age group was divided by the number of person-years within that age group. Mortality rates per person-year of follow-up by country and race were determined. The ethnicity analysis used a breakdown of Allegheny County cohort in Caucasians and African Americans; cases from Havana cohort are referred as Hispanics since information on ethnicity was unavailable for this analysis.

Standardized Mortality Ratio (SMR) was also calculated. Survival analysis and Cox proportional hazard model were included in the analysis.

All analyses were conducted using the computer packages SPSS and Stata.

4.5 University of Pecs, albuminuria follow-up

To examine early microalbuminuria (MA) in children (age<18 years) with Type 1 diabetes, a standardized screening method and 5-year follow-up visits were initiated in 1997 at the University of Pecs, Department of Pediatrics.
Beyond the general metabolic assessment and insulin dose optimization being done during the control visits in the hospital, the children underwent regular complication (e.g. ophthalmologic) examination which was done by the same person with the same methodology throughout the follow-up process.

4.5.1. Clinical evaluation and metabolic investigation

All albumin assays were carried out in the department's central laboratory. Urinary albumin (collected for 24 hour) was measured by immunonephelometry (Turbox nephelometer, reagens from Orion Diagnostica, Finland; linearity: 7-150 mg/l, variability by 12 mg/l: CV= 3.6%, by 128 mg/l: CV=3.9%). Diagnosis of microalbuminuria was based on the results of three consecutive 24-hour urine collections (mean AER ≥ 30mg/day). Median-albuminuria was defined on the individual basis if the mean AER of the three consecutive measures reached the median AER level of the total cohort (≥14.5mg/day).
Glycemic control was assessed by HbA1c measurement using low pressure cation-exchange chronomatography (Bio-Rad DiaSTAT analyzer; measurement range: 4.9-17.2%, reproducibility by 6.4%: CV=2.5%, by 10%: CV=2.1%). Long-term glycemia at follow-up was evaluated using mean HbA1c values from the previous 5 years (4 measures/year, approx. 20 values).
Serum cholesterol and triglyceride levels were determined enzymatically after a 12-hour overnight fast.
Body height and weight were measured in underwear without wearing shoes using Harpenden stadiometer for height and a standard calibrated scale for weight measurements. BMI was calculated as body weight in kilograms divided by height in meters squared. Blood pressure readings were measured by a random-zero sphygmomanometer after a five-minute rest period. Daily insulin requirement was calculated as total insulin dose divided by the body weight (U/kg). Pubertal staging was performed based on the Tanner-scores (1-5).

4.4.2. Statistical analyses

Two-sided Student's t-test and paired t-test (for longitudinal analysis in those patients attended both follow-up visits) were used to examine differences in normally distributed continuous variables. Chi square test and trend analysis were applied to reveal differences in categorical variables. Pearson's correlation was used to assess univariate associations between variables. Logistic regression modeling was used to examine relationships between MA and median-MA status and independent variables.

5. Results

5.1. Mortality and underlying causes of death in the Pittsburgh Metropolitan Area

5.1.1. Living status and demographic characteristics

The living status of 94 percent of the total cases involved in the combined (EDC+DERI study, n=1261) cohort was confirmed (*Table 1.*). There were no statistical differences between the subjects with confirmed living status and missing cases in age at onset ("mean (SD)" 10.1 (4.1) vs 10.7 (4.3) years, respectively), mean year at onset (1971.8 (4.1) vs 1971.8 (4.2), respectively) or sex (50.3% vs 51.3% male, respectively). However, African-Americans were less likely to have verified vital status than Caucasians (84% vs 94%; $p<0.001$). In terms of the demographic characteristics of the study population at follow up, women were significantly younger than men ($p<0.05$). African Americans were also significantly younger than Caucasians ($p<0.05$) and had a significantly shorter duration of diabetes than Caucasians ($p<0.05$). No differences were seen by sex and race in age at onset (10.1 (4.0) years) and in year of diabetes diagnosis (1971.8 (4.2)).

Table 1.
Demographic characteristics of the study population at follow- up (1999) by sex and race
(The combined Children's Hospital of Pittsburgh, PA and the Allegheny County, PA registries)

	Male N=635	Female N=626	Caucasian N=1184	African American N=77	Total 1261
Living status confirmed N (% of total)	595 (94%)	588 (94%)	1118 (94%)	65 (84%)*	1183 (94%)
Died N (% of total)	101 (16%)	99 (16%)	173 (15%)	27 (35%)*	200 (17%)
Demographic variables- mean (SD) shown					
Age at follow-up (years)	36.5 (6.2)	35.7 (6.7)*	36.3 (6.4)	33.7 (7.9)*	36.1(6.5)
Diabetes duration (years)	26.2 (5.4)	25.8 (6.1)	26.1 (5.6)	23.3 (6.9)*	26.0 (5.7)
Age at diabetes diagnoses	10.2 (4.2)	10.0 (3.9)	10.1 (4.0)	10.6 (4.2)	10.1 (4.0)
Year of diabetes diagnosis	1971.8 (4.1)	1971.9 (4.2)	1971.8 (4.1)	1971.7 (4.2)	1971.8 (4.2)
Year of diabetes diagnosis (N, % confirmed dead at follow-up)					
1965- 1969 (n=428)	62 (9.8%)	56 (8.9%)	101 (8.5%)	17 (22.1%)	118 (9.4%)
1970- 1974 (n=446)	28 (4.4%)	30 (4.7%)	50 (4.2%)	8 (10.4%)	58 (4.6%)
1975-1975 (n=387)	11 (1.7%)	13 (2.1%)	22 (1.9%)	2 (2.6%)	24 (1.9%)
Total (n=1261)	101 (16.0%)	99 (15.8%)	173 (14.6%)	27 (35.1%)	200 (15.9%)

* Significant at $p<0.05$

5.1.2. Total (crude) mortality

Overall, 200 deaths were observed (16.8 percent). Those dying were significantly ($p<0.001$) younger, had a shorter duration of diabetes, were older when they developed diabetes and their year of diagnosis was earlier (*Table 2.*). No difference was found by gender, however, African Americans had a greatly increased risk of death (hazard ratio= 3.3, 95% confidence interval: 2.2-5.0) compared to Caucasians.

Table 2. Characteristics of patients by living status and year of diagnosis
(The combined Children's Hospital of Pittsburgh, PA and the Allegheny County, PA registries)

	Alive	Died	1965-69	1970-74	1975-79
n	983	200	427	447	387
Living status confirmed (%)	983 (100)	200 (100)	398 (93.2)	423 (94.6)	362 (93.5)
Males (%)	494 (50.3)	101 (50.5)	217 (50.8)	235 (52.6)	183 (47.3)
African Americans (%)	38 (3.9)	27 (13.5)*	25 (5.9)	28 (6.3)	24 (6.2)
mean (SD) shown					
Age (yrs)	36.9 (5.8)	32.3 (8.2)*	39.2 (6.9)	36.2 (5.9)	32.6 (4.7)*
Diabetes duration (yrs)	27.0 (4.6)	21.0 (7.9)*	29.5 (6.2)	26.1 (4.5)	21.1 (3.2)*
Age at onset (yrs)	9.8 (4.1)	11.3 (3.9)*	9.8 (4.3)	10.1 (3.9)	10.5 (3.9)
Year at onset	1972.3 (4.1)	1969.4 (3.6)*	1967.1 (1.4)	1972.0 (1.4)	1976.8 (1.4)*

* Significant at $p<0.001$

In the Cox regression models, age at onset, diagnosis year, and race predicted total mortality with the hazard ratios of 1.1 (95% CI=1.0-1.3), 0.90 (0.86-0.94), 3.3 (2.2-5.0), respectively (*Table 3.*).

Table 3. Hazard ratios (95% CI) from Cox proportional models for all cause, acute complication and chronic complication mortality
(The Combined Children's Hospital of Pittsburgh, PA and the Allegheny County, PA Registries)

Variable	All cause Mortality (95%CI)	Acute Complications (95% CI)	Chronic Complications (95%CI)
Race	3.3 (2.2, 5.0)	4.9 (2.0, 11.7)	1.1 (.54, 2.0)
Diagnosis year	0.90 (0.86, .94)	1.2 (1.0, 1.3)	1.2 (1.0, 1.4)
Age at diabetes diagnosis	1.1 (1.0, 1.3)	.85 (0.76, 0.93)	1.1 (0.99, 1.3)

5.1.3. Causes of deaths

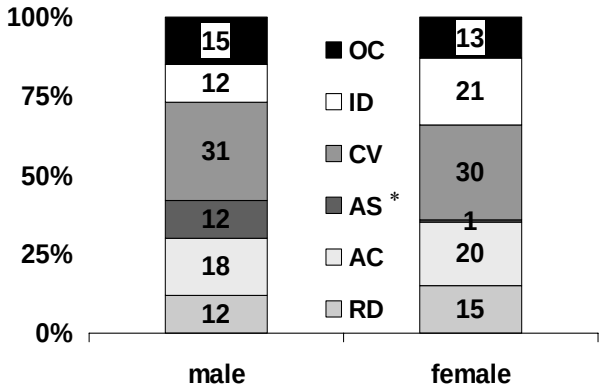
Underlying and secondary causes of death were adjudicated for 82 percent (n=164) of the deceased cases, all of whom had a death certificate. In addition, hospital records (58 percent), coroner and autopsy reports (14 percent), and interview with the next of kin (12 percent) were available. Among the 164 deceased whose causes of death were verified, 19 percent (n=31) died from acute diabetes complications (24 diabetic ketoacidosis, five hypoglycemia, and two unspecified coma), 101 from chronic diabetes related complications (22 renal, 50 cardiovascular, 27 infection and two unspecified diabetes related causes), 15 percent (25) died from other non-diabetes related causes (six from suicide, three from accident, two from drug abuse, six from cancer, two from post-transplant complication, six from other various causes). The cause of death could not be determined in seven cases.

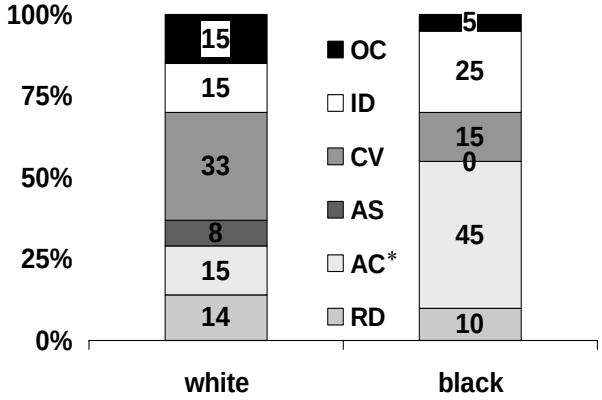
According to the proportionate analysis of cause-specific mortality, the leading cause of deaths was the group of cardiovascular diseases, mainly heart disease and coronary artery disease, followed by acute complications mainly diabetic ketoacidosis. Infection and renal disease played also a big role in the causes of death, while other causes involve diabetes and non-diabetes related problems, post-surgical complications, but mainly cancer (*Figure 1a-b.*).

Figure 1a-b. Proportionate cause-specific mortality by sex and race

(The Combined Children's Hospital of Pittsburgh, PA and the Allegheny County, PA Registries)

all numbers in percentage





*Significant at p<0.01

RD=Renal Disease; AC=Acute Complication; AS=accident/suicide; CV=cardiovascular disease; ID=infection; OC=other causes (cancer, diabetes related, unrelated causes, postsurgical complications)

Similar pattern was seen in the cause-specific mortality rates per 100,000 person-years (*Table 4.*).

Table 4. Cause-specific mortality rates per 100,000 person years (95% CI)
The combined Children's Hospital of Pittsburgh, PA and the Allegheny County, PA registries

Cause of death	n=164	rate/100.000 person-years (95% CI)
Renal disease	22	71.6 (55.0-88.2)
Acute complication	31	100.9 (81.2-120.6)
Accident/suicide/drug abuse	11	35.8 (24.1-47.5)
Cardiovascular disease	50	162.7 (137.7-187.7)
Infection	27	87.8 (69.4-106.2)
Other causes	23	74.8 (57.8-91.7)

5.1.4. Gender differences

There was no difference by gender seen in terms of the acute and various chronic complications (*Figure 1a.*) in the proportionate analysis of causes, however, significantly more deaths from accident/suicide were seen among males ($p<0.01$).

To make more exact analysis about mortality differences by sex and race, twenty-year cause-specific mortality rates per 100,000 person years were calculated for acute, chronic diabetes complications, non-diabetes related and unknown causes (*Table 5.*) Although confidence intervals overlap, males had a somewhat higher mortality from non-diabetes related causes (especially accident/suicide) than females. No other gender differences were seen in cause-specific mortality which is unrelated to race.

Table 5. Twenty-year cause-specific mortality rates (n and %) per 100,000 person years (95% CI) by sex and race
(The Combined Children's Hospital of Pittsburgh, PA and the Allegheny County, PA Registries)

			Gender		**Race**	
Cause of death	N	Overall rate	Male	Female	Caucasian	African American
Acute complications	31	100.7 (65.3, 136.1)	96.0 (47.4,144.6) (n=15)	105.4 (53.8, 157) (n=16)	75.2 (43.8, 106.6) (n=22)	593.2 (205.6, 980.8) (n=9)
Chronic complications	101	328.2 (264.2, 392.2)	307.5 (220.6, 394.4) (n=48)	349.4 (255.4, 443.4) (n=53)	311.2 (247.3, 375.1) (n=91)	659.1 (250.6, 1067.6) (n=10)
Non diabetes related causes	25	81.2 (49.4, 113.1)	115.3 (62.1, 168.5) (n=18)	46.1 (12, 80.2) (n=7)	85.5 (52.1, 119.2) (n=25)	0
Unknown causes	7	22.7 (5.9, 39.5)	19.2 (-2.5, 40.9) (n=3)	26.3 (0.5, 52.1) (n=4)	20.5 (4.1, 36.9) (n=6)	65.9 (-63.3, 195.1) (n=1)
Total deceased	164	532.9 (451.4, 614.4)	538.2 (423.1, 653.3) (n=84)	527.4 (411.8, 643.0) (n=80)	492.1 (412.1, 572.9) (n=144)	1318.4 (1168, 1468.8) (n=20)

5.1.5. Racial differences

The racial distribution of the proportionate mortality approach (*Figure 1b.*) showed a dramatic significant difference between African-Americans and Caucasians in the proportion of acute diabetes complications (45% vs. 15%, respectively).

Same pattern was revealed from the twenty-year mortality analysis. African Americans had a higher mortality from acute complications (mainly ketoacidotic coma) compared to Caucasians (*Table 5.*). The increased mortality from acute complications in African Americans was apparent in both genders though only significant in women, e.g. for women the rate for mortality from acute complications in African Americans was 13 percent compared to 1.2 percent in Caucasians, while for men the difference was 10 percent vs 2.0 percent (*Table 6.*). Racial difference was further examined; no difference was seen by age or duration while the major underlying cause of death from acute complications was diabetic ketoacidosis in both races. The rates regarding the other diabetes-related and non-related causes did not differ significantly between the racial groups.

The proportion of patients whose causes of death were determined based on death certificate alone was somewhat higher among African Americans compared to Caucasians (22 percent vs 12 percent, respectively, $p<0.05$).

Table 6. Twenty-year acute and chronic complication mortality rates (per 100,00 person years and 95%CI) by racial groups (The Combined Children's Hospital of Pittsburgh and the Allegheny County, PA registries)

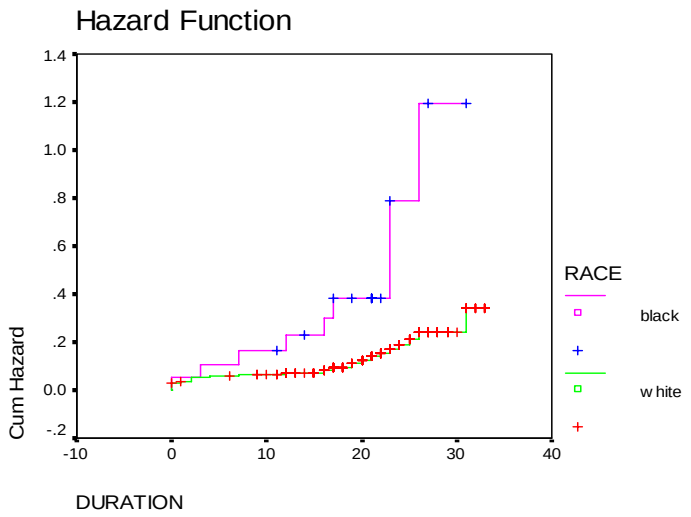
	Caucasian				African American			
Cause of death	Male		Female		Male		Female	
Acute complications	12	80.1 (34.8, 125.4)	10	70.1 (26.7, 113.5)	3	477.7 (-46.8, 1018.2)	6	674.9 (134.9, 1214.9)
Chronic complications	44	293.7 (207, 380.4)	47	329.6(320.2, 423.8)	4	636.9 (12.7, 1261.1)	6	674.9 (134.9, 1214.9)
Total deceased	56	373.8 (280.9, 476.7)	57	399.7 (296, 503.4)	7	1114.6 (288.3, 9371)	12	1349.8 (586.1, 2113.5)

The cumulative hazard curve for acute complication death was significantly worse in African Americans, started separating from the hazard curve of Caucasians already after 10 year of diabetes duration (*Figure 2.*). No difference was seen, however, in the chronic complication hazard curves between the two races.

Figure 2a-b Cumulative hazard for complication deaths by race.

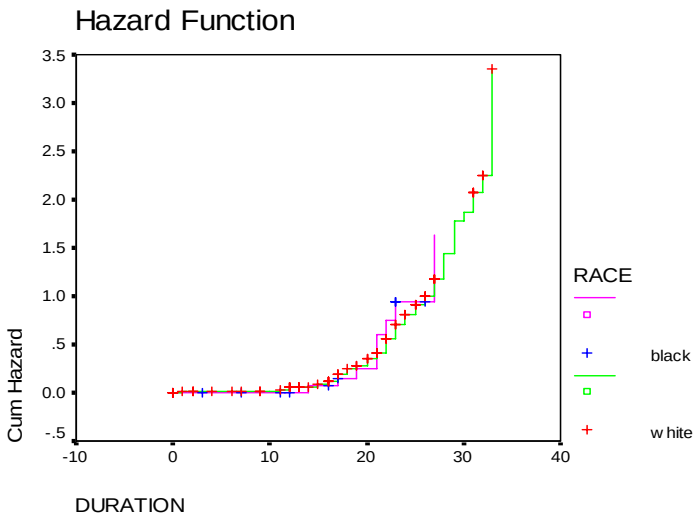
(The Combined Children's Hospital of Pittsburgh, PA and the Allegheny County, PA Registries)

Figure 2/a: Cumulative hazard for acute complication death by race



Log rank p<0.001

Figure 2/b: Cumulative hazard for chronic diabetes complication death by race



Log rank p=0.67

In the Cox regression models, race, age at onset, and diagnosis year predicted total mortality (*Table 7.*). Race and age at diabetes diagnosis were independently predictive for mortality from acute complications, while for mortality from chronic complications, only diagnosis year was predictive (hazard ratio= 1.2: 95% confidence interval: 1.0, 1.4).

Table 7. Hazard ratios (95% CI) from Cox proportional models for all cause, acute complication and chronic complication mortality
(The Combined Children's Hospital of Pittsburgh, PA and the Allegheny County, PA Registries)

Variable	All cause Mortality (95%CI)	Acute Complications (95% CI)	Chronic Complications (95%CI)
Race	3.3 (2.2, 5.0)	4.9 (2.0, 11.7)	1.1 (.54, 2.0)
Diagnosis year	0.90 (0.86, .94)	1.2 (1.0, 1.3)	1.2 (1.0, 1.4)
Age at diabetes diagnosis	1.1 (1.0, 1.3)	.85 (0.76, 0.93)	1.1 (0.99, 1.3)

5.1.6. Temporal trends in cause specific mortality

For analyzing any potential temporal trends in mortality, twenty-year cause specific mortality rates were examined by diagnosis cohort (*Table 8*). Major, significant declines were seen both overall, and for chronic complications alone. Though less marked (i.e. confidence intervals overlap) probably due to lower number of events, acute complications and non-diabetes related causes also showed a similar decline. Though a decreasing trend was apparent for both Caucasians and African Americans for each cause, only the decline in chronic complication mortality rates among Caucasians reached the level of significance reflecting, at least in part, the smaller sample size for African Americans**.**

Table 8. Temporal trends in 20-year cause specific mortality rates (per 100,000 and 95% CI)
(The Combined Children's Hospital of Pittsburgh, PA and the Allegheny County, PA Registries)

	Year of diabetes diagnosis					
	1965-1969		1970-1974		1975-1979	
Cause of death	Number	Rate (95%CI)	Number	Rate (95%CI)	Number	Rate (95%CI)
Acute complications	15	127.8 (63.2, 192.4)	12	108.8 (51, 166.6)	4	50 (1, 99)
Chronic complications	61	519.7 (389.3, 650.1)	29	262.9 (173, 352.8)	11	137.7 (56.4, 219)
Non diabetes related causes	16	136.3 (69.8, 203.1)	6	54.4 (13.5, 95.3)	3	37.5 (-5, 80)
Unknown causes	4	34.0 (0.6, 67.4)	1	9.1 (- 7.6, 25.8)	2	25 (-9.7, 59.7)
Total	96	817.9 (654.3, 981.5)	48	435.3 (312.2, 558.4)	20	250.4 (140.7, 360.1)

5.2. Differences in mortality between Havana and Allegheny County

5.2.1. Living status and demographic characteristics

Overall 887 individuals from the Allegheny County cohort (AC) and 504 individuals from the Havana city cohort (HA) with childhood onset T1D met the criteria for inclusion and therefore were included in the study. The living status of 852 (ascertainment rate of 96.1%) from the AC cohort and 470 (ascertainment rate of 93.3%) from the HA cohort was established as of 1 January, 1991. (*Table 9.*). No differences between registries were seen in men, although women in HA were less likely to have their vital status determined (92% vs. 97%., $p<0.05$).

Table 9. Demographic Characteristics of Studied Population
(US: Allegheny County, PA Registry, 1965-1979 and Cuba: Havana Registry, 1965-1980).

	Males		Females	
	Allegheny County	Havana	Allegheny County	Havana
N	449	259	438	245
Living Status Confirmed	428 (95.3)	244 (94.2)	424 (96.8)*	226 (92.3)*
Age at Onset	9.2 (3.7)	8.8 (3.8)	9.3 (3.4)	8.3 (3.7)
Year of Onset	1971.7 (3.9)	1973.4 (4.4)	1971. 7 (4.1)	1973.1 (4.6)
Mean DM Duration	18.8 (4.3)	16.5 (4.8)	18.6 (4.7)	16.5 (5.0)
Person-years	8,456	4,237	8,156	3,996

*HA vs. AC significant at $p<0.05$

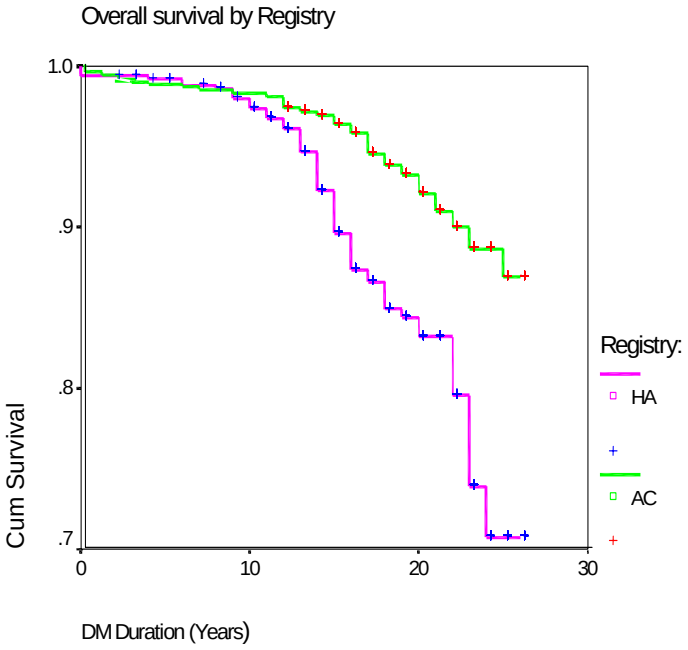
5.2.2. Mortality rates and standardized mortality ratios

Cumulative survival by registry demonstrated a worse survival in HA (84% at 20 years) than AC (91% at 20 years) (*Figure 3.*).

Mortality in HA was higher than in the AC in both genders (males, $p<0.01$; females, $p=0.05$). Mortality rates were higher in HA for both men and women although in women confidence intervals overlapped. SMR were significantly higher in HA in both sexes.

Figure 3. Cumulative survival of subjects by registry

(US: Allegheny County, PA Registry, 1965-1979 and Cuba: Havana Registry, 1965-1980).



AC: Allegheny County registry; HA: Havana City Registry

5.2.3. Causes of deaths

When underlying causes of deaths (*Table 10*) were examined, major differences in cause specific mortality were found. More deaths in AC were attributed to acute complications than to any other cause, while in HA the leading cause of death was nephropathy, which accounted for nearly half of the deaths. Interestingly the mortality rate for acute complications was lower in HA than in AC (although this difference was not significant) while renal mortality was eight times higher in HA than in AC (430/100,000 vs. 53 /100,000 person-years). There was an excess mortality among African-Americans attributed to acute complications (406/100,000) and infections (271/100,000) when compared to the Hispanics of HA or the Caucasians of AC.

Table 10. Cause specific mortality rate (x 100,000 person-years) by country and race. (US: AC, PA Registry, 1965-1979 and Cuba: HA Registry, 1965-1980).

Causes of Death	Caucasians		Allegheny County African-Americans		Total		Cuba Hispanics	
	Rate	%	Rate	%	Rate	%	Rate	%
Nephropathy	44	12.5	172	18.2	53	13.4	430	48.6
95%-CI	21-108		37-1,765		28-113		313-608	
Acute Complications	108	25.0	406	36.4	128	26.9	66	7.1
95%-CI	63-193		150-1,473		82-213		28-196	
CVD	73	19.6	-	-	67.7	16.4	130	14.3
95%-CI	41-143		-		38-133		72-262	
Infections	65	17.9	271	27.3	79	19.4	136	14.3
95%-CI	35-135		86-1,297		46-147		75-276	
Other	87	21.4	-	-	81	17.9	149	16.0
95%-CI	50-164		-		46-152		85-289	
Unknown	13	3.6	172	18.2	24	6.0	-	-
95%-CI	3-141		36-1,799		9-88			
Total	390.	100.0	1,022	100.0	434	100.0	911	100.0
95%-CI	302.1-512.2		591-1,919		344-556		733-1,147	

5.2.4. Gender differences

Gender differences were not seen in the demographic characteristics of each cohort, nor in the mortality rates and standardized mortality ratios (*Table 11.*).

Table 11. Age-Sex-Adjusted Mortality Rate and Standardized Mortality Ratios by Gender (US: AC, PA Registry, 1965-1979 and Cuba: HA Registry, 1965-1980).

	Males		Females	
	AC	HA	AC	HA
N	449	259	438	245
Deceased (%)[1]	29 (7)	37 (14)	38 (9)	33 (14)
Mortality Rates X 100,000	368	925	503	897
95%-CI	259-539	687-1,275	369-703	655-1,262
SMR	342	858	378	882
95%-CI	236- 513	618-1,228	266-556	670-1,274

[1] AC vs. HA p< 0.01 for males; p=0.05 for females; Caucasians vs. African Americans p<0.01; African American vs. Hispanics p=0.44.

Based on the Cox proportional hazard model (*Table 12.*), the risk of death was not associated to gender (OR=1.1, 95% CI: 0.8-1.5, p=0.668).

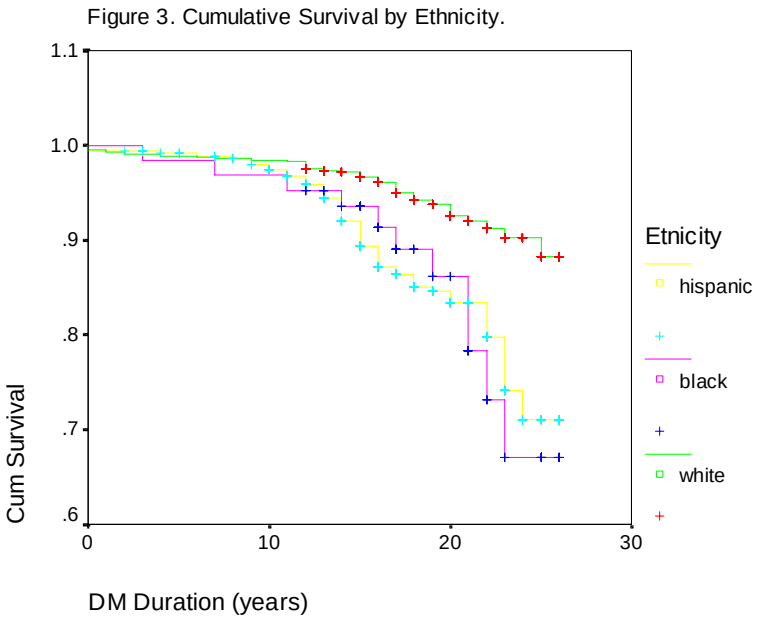
Table 12. Cox Proportional Hazard Model for all cause mortality. (US: AC, PA Registry, 1965-1979 and Cuba: HA Registry, 1965-1980).

Factor	**OR**	**95%-CI**	**p**
Sex, Male:Female	1.1	0.8-1.5	0.668
Race, US Caucasians:US African Americans	2.8	1.5-5.4	0.002
Race, US Caucasians:Cuban Hispanics	3.3	2.2-5.0	<0.001
Year of Diagnosis	1.1	0.8-1.3	0.548
Year of Birth	0.9	0.9-0.6	0.435
Age at Diagnosis	0.9	0.6-1.4	0.652

5.2.5. Racial differences

The cumulative survival analysis of subjects by race (*Figure 4.*) suggests that mortality in AC for African-Americans was worse than for Caucasians but similar to that seen in HA for Hispanics.

Figure 4. Cumulative survival of subjects by race
(US: Allegheny County, PA Registry, 1965-1979 and Cuba: Havana Registry, 1965-1980).



Same pattern was seen when adjusted mortality rates and SMRs were analyzed (*Table 13.*).

Table 13. Age-Sex-Adjusted Mortality Rate and Standardized Mortality Ratios by Race
(US: AC, PA Registry, 1965-1979 and Cuba: HA Registry, 1965-1980).

	Race		
	AC		HA
	Caucasian	African-American	Hispanic
N	823	63	504
Deceased (%)*	56 (7)	11 (18)	70 (14)
Mortality Rates x 100,000	390	1,022	912
95%-CI	302-512	591-1,919	733-1,147
SMR	285	922	840
95%-CI	215-385	515-1,820	665-1,077

*Caucasians vs. African Americans p<0.01; African American vs. Hispanics p=0.44.

Results of the Cox proportional hazard model for all cause mortality indicated that after adjusting for age, gender, year and age of diagnosis the Odds Ratio was 3.3 (95%-CI=2.2-5.0, p<0.001) times higher for Hispanics than for Caucasians (*Table 12.*). For African-Americans the risk of any cause mortality was 2.8 (95%-CI=1.5-5.4, p=0.002) times higher compared to Caucasians. The risk of death was not associated with sex, year of diagnosis, year of birth or age at diagnosis, after controlling for ethnic group.

5.2.6. Effect of age at onset and year of diagnosis

Mortality in HA was higher irrespective of age at diagnosis (*Table 14.*) though this was not statistically significant among those with onset 0-4 years of age (p=0.08 for 0–4 years; p<0.01 for 5–9 years; p<0.01 for 10–14 years). The difference in crude mortality and SMR between the Havana and Allegheny County cohort became even more dramatic in the 1970s (p=0.28 for 1965–1970; p<0.01 for 1971–1980.), however, a nice decreasing trend in the standardized mortality ratios could be seen in both cohorts with the later years of diagnosis.

Table 14. Mortality Rates and Standardized Mortality Ratios by Age at Diabetes Onset and Diabetes Diagnosis (US: Allegheny County, PA Registry, 1965-1979 and Cuba: Havana Registry, 1965-1980).

	Age at Diagnosis						Year of Diagnosis			
	0-4		5-9		10-14		65-70		71-80	
	AC	HA	AC	HA	AC	HA	AC	HA	AC	HA
N	105	95	306	178	476	231	367	148	520	356
Deceased* (%)	5 (5)	11 (12)	17 (6)	25 (14)	45 (9)	34 (15)	45 (12)	32 (22)	22 (4)	38 (10)
Mean Survival Time	25.2	24.5	25.2	23.7	24.6	23.3	24.9	23.9	19.5	18.9
95%-CI	24.5-26.0	23.6-25.3	24.8-25.5	22.9-24.5	24.2-25.0	22.4-24.1	24.5-25.2	23.2-24.6	19.3-19.7	18.6-19.3
Crude	258	679	328	886	562	1,056	594	1,162	295	777
95%-CI	109-765	395-1,271	208-548	618-1,315	425-761	755-1,477	449-901	851-1,626	197-464	577-1,072
SMR	149	612	263	901	492	980	547	1,141	185	715
95%-CI	46-740	336-1,239	158-472	615-1,374	362-685	695-1,427	408-752	814-1,649	111-333	515-1,022

Background mortality for Standardized Mortality Ratios calculation: general US population for Allegheny County and the Cuba general population for Havana.

*AC vs. HA: $p = 0.08$ for 0–4 years; $p < 0.01$ for 5–9 years; $p < 0.01$ for 10–14 years; $p = 0.28$ for 1965–1970; $p < 0.01$ for 1971–1980.

5.3. Incidence of coronary artery disease (CAD) and nephropathy (MA, ON, ESRD)

5.3.1. DERI complication survey

Out of the 1075 individuals who comprised the Allegheny County registry (DERI-USA cohort), living status of 975 (91 %) and complication status of 788 (73%) for CAD and 798 (74%) for ESRD was ascertained as of 1 January 1999. There was no statistically significant differences in frequencies by year of onset (p=0.16) or sex (p=0.70) between those traced and those missing on 1 January, 1999, however, mean age at onset was higher (11.5±4.2 (±SD) year vs. 10.7±4.1 year, p=0.003) and the proportion of Caucasians was lower (88 vs. 94%, p=0.007) in those not traced.

Coronary artery disease:

During the observational period, 63 (8%) subjects developed CAD. Mean age at diabetes onset was 10.4±4.2 (±SD) while mean diabetes duration at death from CAD or censoring was 24.9±5.8 years. Incidence density was calculated as 325/100,000 person-years. Cumulative incidence rate of CAD was determined as 9.1% at 25 years, while other rates were 0.7% at 15; 3.0% at 20; and 28.1% at 30 years, respectively. No differences by gender or race were seen in the incidence rates (*Figure 5a-b.*).

Figure 5a. Cumulative incidence rate of CAD by gender
(DERI-USA complication survey: Allegheny County, PA Registry, 1965-1979)

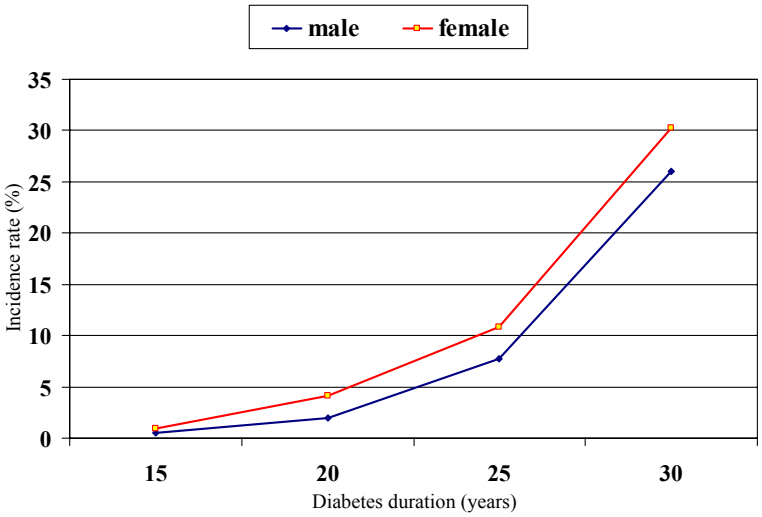
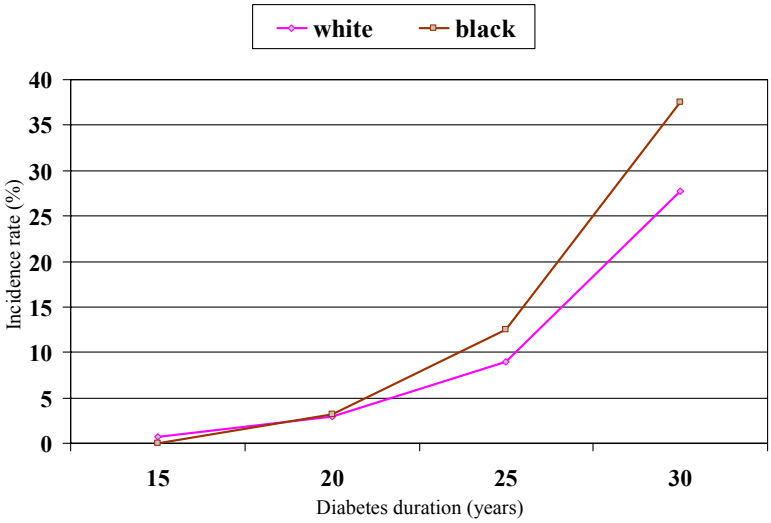


Figure 5b. Cumulative incidence rate of CAD by race

(DERI-USA complication survey: Allegheny County, PA Registry, 1965-1979)



When sub-cohorts by year of diabetes onset (1965-69, 1970-74 and 1975-79) were compared, the mean age at onset (11.5 ± 5.6 yrs) for subjects diagnosed between 1975-79 was significantly (p=0.01) higher than for those diagnosed between 1965-69 (10.6±4.4 yrs) and 1970-74 (10.7±4.1 yrs), however, differences were not seen in sex and race. The cumulative incidences of CAD were not different significantly between patients diagnosed in earlier calendar years and those diagnosed more recently (*Table 15.*).

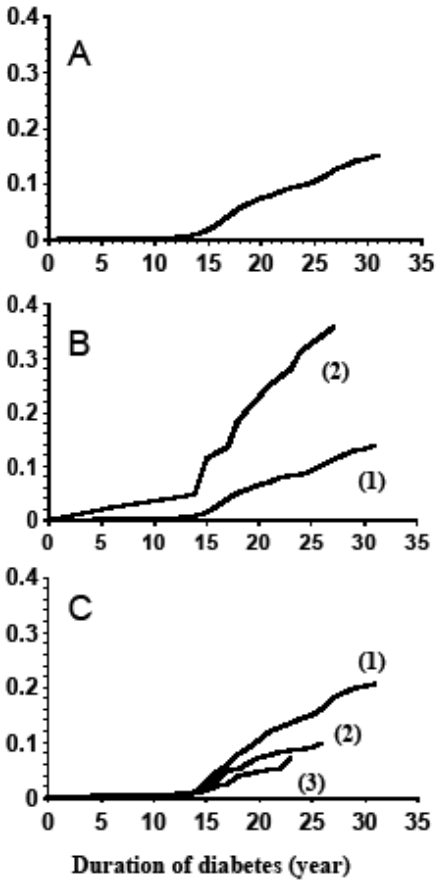
Table 15. Cumulative incidence rates of coronary artery disease (CAD) by the year of diabetes diagnosis
(DERI-USA complication survey: Allegheny County, PA Registry, 1965-1979)

	1965-69		**1970-74**		**1975-79**	
	CAD (n)	Incidence rate (%)	CAD (n)	Incidence rate (%)	CAD (n)	Incidence rate (%)
at 20 yrs	6	3,0%	7	2,8%	7	3,1%
at 25 yrs	15	7,6%	16	6,9%	9	
at 30 yrs	24	14,1%	24		9	

End-stage renal disease:

During the observation, 104 individuals (13.0%) developed ESRD. Mean duration of diabetes at death or censoring was 25.0 ± 5.6 ($\pm$ SD) years. The incidence rate of ESRD was 521/100,000 person-years. Cumulative incidence rate of ESRD was 11.3 % at 25 year of diabetes (*Figure 6a.*). The 20-year cumulative incidence rates of ESRD for the subjects diagnosed between 1965-69, 1970-74 and 1975-79 were 9.1%, 4.7% and 3.6% respectively, and differences between 1965-69 vs. 1970-74 (p=0.0073) and 1965-69 vs. 1975-79 (p=0.0091) were statistically significant (p value for 3 survival curves=0.006) (*Figure 6c.*). The 20-year cumulative incidence rate of ESRD in African Americans was statistically higher than that of Caucasians (21.9% vs. 5.2%: p<0.0001) (*Figure 6b*).

Figure 6a-c. Cumulative incident rates (n/100) of end-stage renal disease (ESRD)
(DERI-USA complication survey: Allegheny County, PA Registry, 1965-1979)



A: overall,

B: Caucasian (1) vs. African-American (2) ($p<0.0001$),

C: diagnosed between 1965-69 (1), 1970-74 (2) and 1975-79 (3) ($p=0.006$)

Cox proportional modeling indicated that a lower age at onset (OR=0.90/year, 95% CI: 0.85-0.95, p=0.001), a diagnosis in recent years (OR=0.55 (1970-74 vs. 1965-69), 95% CI: 0.34-0.88, p=0.013, and OR=0.39 (1975-79 vs. 1965-69), 95% CI: 0.20-0.74, p=0.0041) and being Caucasian (OR=0.60 (Caucasian vs. African-American), 95% CI: 0.46-0.77, $p<0.0001$) were protective against ESRD.

5.3.2. EDC complication follow-up

After excluding baseline cases (n=53) and those with no follow-up (n=19), 15% (56/382) of individuals developed new onset coronary artery disease (CAD) during the 6-year follow-up (see baseline characteristics in *Table 16.*). Similarly, 29 incident cases (10%) of overt nephropathy (ON) were diagnosed out of 294 eligible subjects. During complication follow-up with a total of 2113 person-years for CAD and 1620 person-years for ON, somewhat

higher incidence density was seen for CAD than ON (24.6/1000 person-years vs. 17.3/1000 person-years).

5.3.3. University of Pecs, albuminuria follow-up

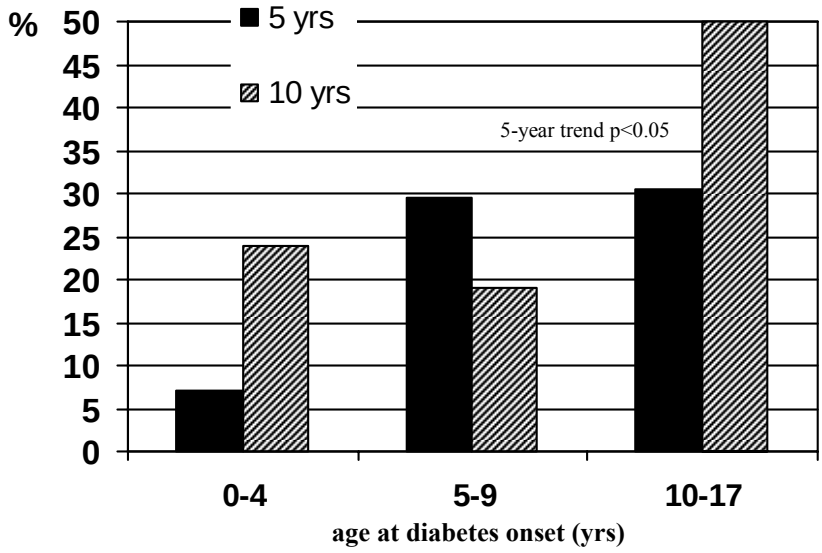
5- and 10-year cross-sectional results of 107 patients with childhood (<18 years) onset Type 1 diabetes were examined. Mean age at onset of the 44 boys and 63 girls was 6.4±3.9 (±SD) years. 88 children (35 boys and 53 girls) examined on the 5-year follow-up visit while only 44 (18 boys and 26 girls) could be reached at 10 years for the hospital follow-up.

Results showed a microalbuminuria (MA) incidence of 18 percent (n=19/107) at 5 years of diabetes duration, however, 39% of subjects developed median-albuminuria (≥14.5mg/day). The 10-year cumulative incidence rate of MA was found to be 23% (10/44). There was no difference seen in gender regarding the incidence rates at 5 and 10 years.

The 5-year incidence of MA was found to be significantly lower (chi square trend $p<0.01$) among patients with early childhood (0-4 years) onset T1D compared to those in higher age groups (5-9, 10-17 yrs) by diabetes onset (*Figure 7*).

In the 5-year longitudinal analysis, progression from normoalbuminuria to MA was revealed in 30% of patients, while improvement from MA to normoalbuminuria was detected only in two cases (7.4%).

Figure 7. Cumulative incidence of MA at 5 and 10 years by age at diabetes onset



5.4. Risk factors and predictors of coronary artery disease (CAD) and nephropathy (MA, ON)

5.4.1. EDC complication follow-up

Baseline risk factors by CAD and ON incidence status at 6-year follow-up were analyzed (*Table 16.*).

Compared to participants who remained free of CAD, those who developed the disease had significantly higher age, duration, HbA1, total and LDL cholesterol, triglycerides, systolic blood pressure, Beck depression score, white blood cell count (WBC), fibrinogen and lower estimated glucose disposal rate (eGDR - a good estimation of insulin sensitivity).

HbA1 and triglycerides were statistically higher in incident cases of ON compared to non-cases, although again, eGDR was lower in those who developed overt nephropathy. Significantly more hypertensive individuals were found among both CAD and ON cases then in those did not develop the complications. However, the number of smokers was significantly higher only among ON cases compared to non-cases. Among the components of the fibrinolytic system, PAI-1 values were lower while tPA-PAI-1 values were higher in incident CAD compared to the non-cases, though the trends were non-significant. However, after adjustment for age, PAI-1 correlated positively ($r=0.02$) with CAD status. For incident ON, both PAI-1 and tPA-PAI-1 were higher in those who developed the complication, although only the latter difference was significant. This association became somewhat stronger ($p=0.02$) when only those were analyzed who did not develop CAD simultaneously.

Table 16. Baseline risk factors and markers by complication incidence status
(Pittsburgh Epidemiology of Diabetes Complications Study: 6-yr follow-up (1990-1992 to 1996-1998))

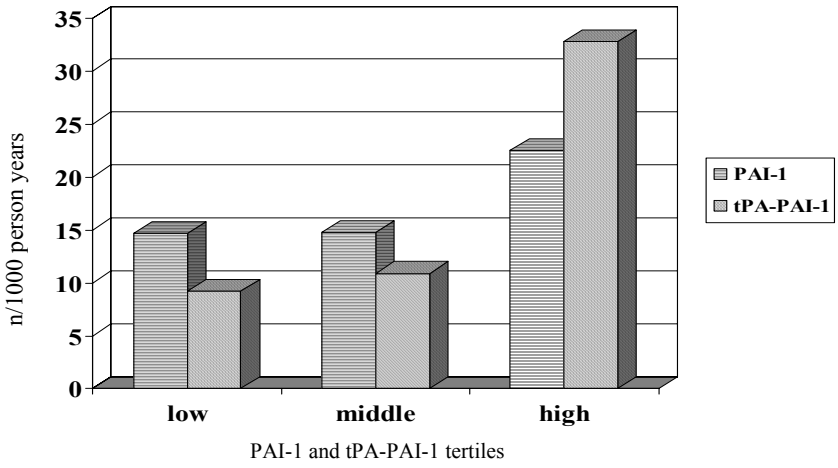
WHR: waist to hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure; WBC: white blood cell count; eGDR: estimated Glucose Disposal Rate; HTN: hypertension

	CAD (n=56)	**Non-CAD (n=326)**	**P-value**	**ON (n=29)**	**Non-ON (n=265)**	**P-value**
Age (yrs)	36.1±7.1	30.1±7.4	*<0.001*	31.5±6.7	31.1±8.1	0.74
Duration (yrs)	28.4±7.3	21.5±6.9	*<0.001*	21.5±7.7	22.7±7.6	0.43
BMI (kg/m2)	24.5±3.6	24.2±3.2	0.52	23.4±3.2	24.4±3.2	0.13
WHR*	0.83±0.07	0.81±0.07	0.08	0.82±0.07	0.81±0.07	0.48
HbA1 (%)	11.2±1.6	10.7±1.7	*0.03*	11.7±2.0	10.5±1.5	*0.008*
Cholesterol (mmol/L)	5.34±1.0	4.71±1.1	*<0.001*	5.08±1.2	4.66±0.8	0.07
HDL-chol (mmol/L)	1.30±0.3	1.37±0.3	0.17	1.34±0.3	1.34±0.3	0.87
LDL-chol (mmol/L)	3.32±0.8	2.90±0.9	*0.001*	3.10±0.9	2.85±0.7	0.16
TG (mmol/L)	1.45±0.9	1.00±0.7	*<0.001*†	1.32±0.9	0.97±0.6	*0.04*†
SBP (mmHg)	122±16	112±14	*<0.001*	110±14	111±12	0.87
DBP (mmHg)	74±12	71±9	0.09	73±8	70±8	0.10
Beck score	8.4±7.7	5.3±5.3	*0.006*‡	8.0±7.5	5.5±5.8	0.06‡
WBC (x109/L)	7.6±1.9	6.9±2.1	*0.01*†	7.1±1.7	6.9±2.1	0.43†
eGDR (mg/kg/min)	6.6±2.3	7.8 ±1.7	*0.001*	6.2±2.1	8.1±1.5	*<0.001*
HTN (%)	34	12	*<0.001*	31	7	*<0.001*
Smoking (%)	22	15	0.24	28	13	*0.05*
Fibrinogen (mg/dL)	327 ±86	291 ±96	*0.006*	340 ±147	284 ±90	0.06
PAI-1 (ng/mL)	23.8±16.9	26.1±17.3	0.39†	28.3±17.9	25.3±17.0	0.45†
tPA-PAI-1 (ng/mL)	1.48±0.7	1.40±1.0	0.38†	1.68±0.8	1.41±1.0	*0.04*†

* determined two years earlier; † log transformed before testing; ‡ non-parametric test performed

The incidence densities of CAD and ON by PAI-1 levels did not reveal any significant trend for either measure. However, a significant trend ($p<0.01$) for an increased incidence of ON by tPA-PAI-1 tertiles was seen (*Figure 8.*).

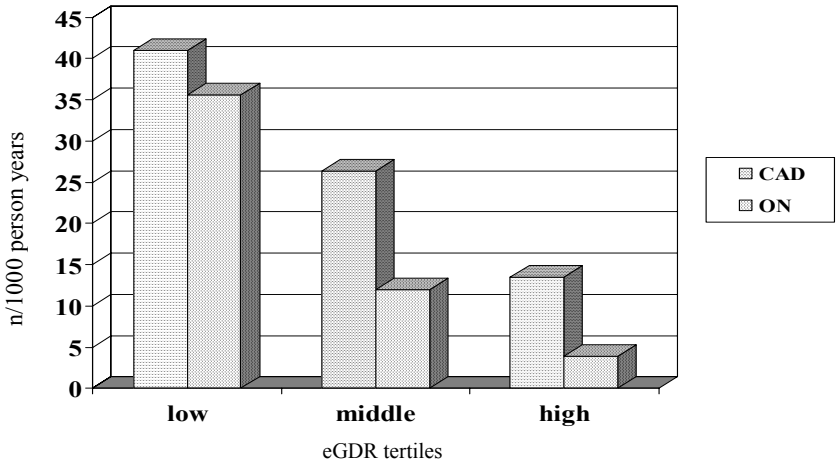
Figure 8. Incidence density of overt nephropathy by plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA)-PAI-1 levels.



significant trend ($p<0.01$) by tPA-PAI-1 tertiles

No difference was seen in the trends between males and females. A significant decreasing trend in incidence of both CAD and ON by eGDR tertiles was observed ($p<0.01$) showing univariate association between the complications and insulin resistance (*Figure 9.*).

Figure 9. Incidence density of coronary artery disease (CAD) and overt nephropathy (ON) by tertiles of estimated glucose disposal rate (eGDR - an estimation of insulin resistance)



significant trend ($p<0.01$) for CAD and ON by eGDR tertiles

In Cox regression models the following variables were available for modeling: age, HbA1, triglycerides, hypertension, Beck score, WBC, eGDR, fibrinogen, PAI-1 and tPA-PAI-1. In the Cox model only age (RR=1.05, 95% CI: 1.0-1.2, p=0.025) and triglycerides (RR: 14.2, CI: 1.5-22.2, $p<0.001$) were independent predictors of CAD while in the case of ON, HbA1 (RR: 1.4, CI: 1.1-1.9, p=0.022) and eGDR (RR: 0.7, CI: 0.3-0.9, p=0.001) predicted the development of complication.

5.4.2. University of Pecs, albuminuria follow-up

5- and 10-year cross-sectional and 5-year longitudinal follow-up results of 107 children with Type 1 diabetes were examined.

5-year cross-sectional data showed significant correlation between albuminuria and age ($r=0.23$, $p<0.05$). Patients with MA were significantly older (*Table 17.*) and they had significantly higher Tanner-score ($p<0.01$).

Table 17. Characteristics of patients at 5 years of diabetes duration by albuminuria status

	Microalbuminuria	Normoalbuminuria	p-value
N	19	66	
Age (years)	14.6±3.6	11.0±3.8	0.001
Tanner-score	3.7±1.4	2.5±1.6	<0.01
BMI (kg/m^2)	20.6±6.6	20.0±4.7	0.72
Systolic blood pressure (mmHg)	109.8±18.0	105.7±15.1	0.38
Diastolic blood pressure (mmHg)	69.7±11.6	69.2±12.1	0.88
Cholesterol (mmol/l)	4.5±0.9	4.5±0.8	0.80
Triglyceride (mmol/l)	0.9±0.5	0.9±0.7	0.63
Insulin/kgbw (U/kg)	0.9±0.3	0.9±0.2	0.81
Insulin shots (n/day)	4.5±0.8	4.4±0.9	0.55
HbA1c (%)	8.7±1.9	8.9±1.4	0.68

mean ± SD

Beyond the above differences, those with median-albuminuria had significantly higher BMI and systolic blood pressure values ($p<0.02$) compared to the normoalbuminuric children. Individuals with higher Tanner-score (=4-5; more mature during puberty) had an almost 3-fold bigger chance (OR=2.7, 95% CI: 1.0-7.8, p=0.05) for developing MA than children with lower maturity degree (Tanner-score: 1-3).

In multivariate analysis only age was proved to be an independent predictor of MA or median-albuminuria (RR=1.4, 95% CI: 1.1-1.8; RR=1.4, 1.1-1.7, respectively).

Significantly higher HbA1c ($p<0.01$), systolic blood pressure ($p<0.001$), insulin requirement ($p=0.01$) and Tanner-score ($p<0.001$) was found at 10 years compared to the previous visit at 5 years. Markedly higher HbA1c, systolic blood pressure, insulin requirement and Tanner-score was detected in subjects with progression from normoalbuminuria to MA, however, the differences were not statistically significant (*Table 18.*). The mean Tanner-score was found to be significantly higher ($p<0.05$) among children with an albuminuria progression of 14.5mg/day during the 5-year follow-up.

Table 18. Characteristics of patients by 5-year progression to microalbuminuria
(incident cases: progression to MA between the 5-yr and 10-yr visit)

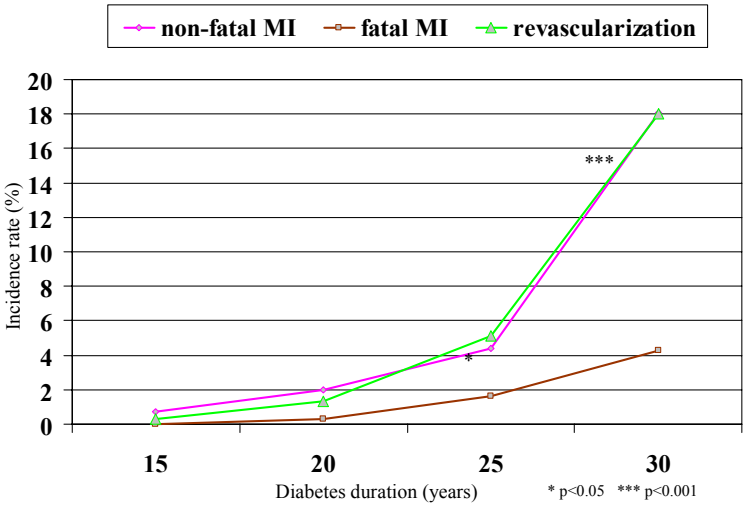
	Incident cases	**Non-cases**
N	8	19
Age (years)	15.2±2.8	15.5±3.2
Tanner-score	4.4±0.9	3.7±1.6
BMI (kg/m^2)	21.3±2.5	21.5±3.1
Systolic blood pressure (mmHg)	122.1±14.8	118.2±12.3
Diastolic blood pressure (mmHg)	71.1±8.2	72.4±10.9
Cholesterol (mmol/l)	4.5±0.8	4.6±0.9
Triglyceride (mmol/l)	0.9±0.4	1.1±0.5
Insulin/kgbw (U/kg)	1.1±0.3	0.9±0.2
Insulin shots (n/day)	4.6±0.5	4.5±0.6
HbA1c (%)	10.4±2.3	9.9±1.2

Non-significant differences ($p>0.05$)

5.5. Survival after coronary artery disease (CAD) and revascularization (RV) therapy

Among those patients who developed CAD in the DERI-USA, Allegheny County cohort (see section 5.3.1.) 35 subjects developed non-fatal myocardial infarction (MI), 41 underwent some form of revascularization (percutan transluminary coronary angioplasty (PTCA) in 20 cases, coronary artery bypass grafting (CABG) in 12 cases and 9 patients underwent both) and 8 individuals died from CAD. The incidence rates of non-fatal MI and revascularization increased dramatically after 25-year of diabetes duration, while the incidence of fatal MI remained significantly lower compared to non-fatal MI (*Figure 10*).

Figure 10. Cumulative incidence rate of coronary artery disease by its clinical manifestation
(DERI-USA complication survey: Allegheny County, PA Registry, 1965-1979)



MI: myocardial infarction

Revascularization: percutan transluminary coronary angioplasty and/or coronary artery bypass grafting

Cumulative survival of subjects with myocardial infarction followed by revascularization was 76% at 5 years, (75%, in cases treated by PTCA only, 87.5%, with CABG and 60% of those underwent both forms of RV) (*Table 19.*). However, significantly less ($p<0.01$), only 53% of patients who developed MI but not treated with any form of revascularization survived 5 years. Patients who underwent PTCA or CABG alone, regardless whether they developed myocardial infarction, had a significantly higher survival rate compared to those who developed MI but did not have any form of RV ($p=0.003$, $p=0.04$, respectively).

Table 19. 5-year cumulative survival of CAD by revascularization therapy (DERI-USA complication survey: Allegheny County, PA Registry, 1965-1979)

	No. of cases	No. of deaths	death rate (%)	5-year survival rate
no MI, no RV	725	76	10,5%	
MI without RV	19	10	52,6%	52,6%
MI followed by PTCA	8	2	25,0%	75,0%
MI followed by CABG	8	2	25,0%	87,5%
MI followed by PTCA+CABG	5	4	80,0%	60,0%
PTCA without MI	16	2	12,5%	100,0%
CABG without MI	7	1	14,3%	100,0%
Any RV without MI	20	2	10,0%	100,0%

MI: myocardial infarction; RV: revascularization therapy; PTCA: percutan transluminary coronary angioplasty
CABG: cononary artery bypass grafting

5.6. Survival after end-stage renal disease (ESRD) and renal replacement therapy (RRT)

Among those individuals who developed ESRD (n=104) in the DERI-USA, Allegheny County cohort (see section 5.3.1.), 29 patients (28%) received dialysis alone, 44 patients (42%) underwent dialysis followed by kidney transplantation, 26 (25%) underwent successful transplantation alone and 5 individuals (5%) had a failed kidney transplantation followed by dialysis therapy. In the group with dialysis therapy followed by kidney transplantation, mean duration between introduction of dialysis and transplantation was 1.5±1.6 (±SD) years. Of 75 renal transplant recipients, information from 60 patients was available, as was whether they received pancreatic-kidney transplantation. Nineteen subjects underwent pancreatic-kidney transplantation.

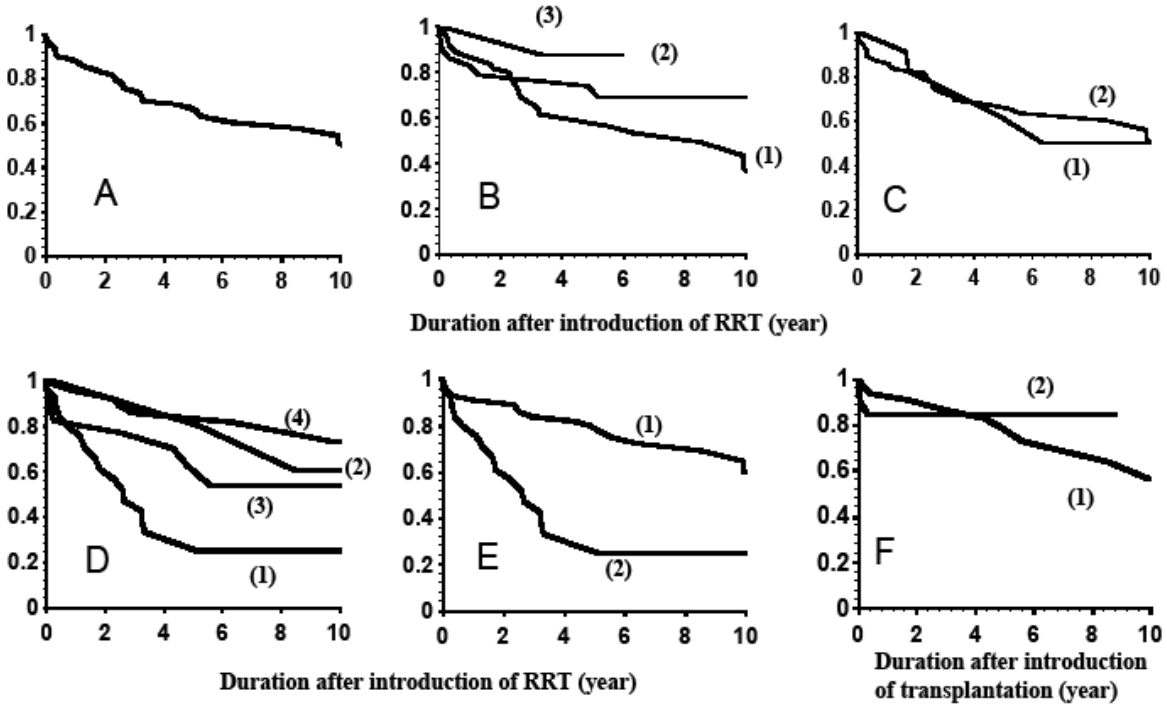
Cumulative survival rates after introduction of RRT were 66.5% at 5 years and 51.2% at 10 years, respectively (*Figure 11a*.).

The 5-year cumulative survival rates after introduction of RRT of the subjects diagnosed between 1965-69, 1970-74 and 1975-79 were 58.8%, 73.5% and 87.5% respectively (*Figure 11b*), however, the differences did not reach significance (p=0.07).

Cumulative survival rates at 5 years by groups of dialysis therapy alone, dialysis followed by kidney transplantation, successful transplantation alone and failed kidney transplantation followed by dialysis therapy were 32.8 %, 85.8%, 62.0% and 80% respectively (*Figure 11d.*). The difference between dialysis therapy alone and dialysis followed by kidney transplantation reached significance (p<0.0001 and for 4 survival curves, p=0.0002). Cumulative survival rate of all transplant recipient was 79.6% at 5 year, which was significantly higher than that of the subjects of dialysis therapy alone (32.8%, p=0.0002) (*Figure 11e*). Survival of pancreatic-kidney transplantation recipients initially tended to be worse, but was subsequently better, although the overall difference did not reach significance (p=0.7) (*Figure 11f*).

Cox modeling revealed that the relative risk of death for all transplantation recipients against dialysis therapy alone was 0.25 (95% CI: 0.13-0.47; p<0.001). Among groups of dialysis followed by kidney transplantation, successful transplantation alone and failed kidney transplantation followed by dialysis therapy, only dialysis followed by kidney transplantation had significantly lower risk of death compared to dialysis therapy alone. In all transplant recipients, the relative risk of death for pancreatic-kidney transplantation recipients compared to those with kidney transplantation only was 0.51 (95% CI: 0.14-1.8; p=0.29).

Figure 11a-f. Cumulative survival rates after introduction of Renal Replacement therapy (RRT)
(DERI-USA complication survey: Allegheny County, PA Registry, 1965-1979)



A: overall

B: diagnosed between 1965-69 (1), 1970-74 (2) and 1975-79 (3) (P=0.073)

C: Caucasian (1) vs. African-American (2) (P=0.8)

D: dialysis alone (DA: 1), dialysis followed by kidney transplantation (DK: 2),
successful transplantation alone (TA: 3), failed kidney transplantation followed by dialysis (KD: 4)
(DA vs. DK: p<0.0001, overall: p=0.0002)

E: all transplantation recipient (1) vs. dialysis only (2) (P=0.0002)

F: kidney transplantation (1) vs.pancreatic kidney transplantation (2) (P=0.7)

6. Discussion

6.1. Cause-specific mortality rates in the Pittsburgh Metropolitan Area

Our data are based on a combined analysis of both population (Allegheny County, Pennsylvania) and hospital (Children's Hospital of Pittsburgh) based registries of childhood onset Type 1 diabetes. The majority of patients came from the Allegheny County Registry, were diagnosed at an older age ($p<0.05$) but had similar diabetes duration compared to those coming from the hospital registry. This pattern is consistent with a bias for younger cases at onset to be referred to the specialist Children's Hospital.

The inadequacy of death certificate classification alone has long been recognized, both generally and in Type 1 diabetes (*West KM, 1979*). The application of the standardized procedures has led to a major redistribution of the causes of death (*DERI Study Group 1991*). These changes resulted from both the review process and the inappropriate completion of the original death certificate (in many cases the last mentioned item was clearly not intended to be the underlying cause of death). Overall, in our analysis only 16 percent of the cases were decided by death certificate alone which contributed significantly to the accuracy of the results. Furthermore, the DERI mortality classification process itself provided a highly standardized, unique method to make more appropriate conclusions from cause-specific mortality data. Without the mortality classification committee review, the proportion of cases due to acute complications and kidney disease would have been seriously underestimated. The use of death certificates alone would have been too imprecise to determine differences in cause-specific mortality data across populations.

Data from international studies also indicate that the proportion of deaths due to acute complications may have been seriously underestimated, and that acute complications may account for 33 percent of deaths among individuals with Type 1 diabetes within the first 20 years (*DERI Study Group Diabetes Care 1991*) and were either the first or the second leading cause of death in patients from Finland, Japan and Israel (*DERI Study Group Diabetes Care 1991*) in the early years. Other studies investigating mortality of Type 1 diabetes in children with 20 years of onset showed that one-third to one-half died of acute complications, mostly due to diabetic ketoacidosis (Laing *SP 1999, Scibilia J 1986, Hirasing RA 1995*). A substantial number of deaths may occur at the onset of the disease (*Thordarson H 1995*), suggesting that delayed diagnosis may contribute to excess mortality.

In our current analysis, however, only five children died from acute complications within one year of diagnosis so this alone cannot explain the difference. Overall, in the current report, we found that 45 percent of African Americans and 15 percent of Caucasian deaths resulted from acute complications.

Regarding deaths from chronic diabetes complications, our findings are consistent with the literature showing cardiovascular diseases and nephropathy are responsible for most premature mortality in people with 26-year long Type 1 diabetes. A pervious analysis of the Allegheny County cohort reported that 2% of people with childhood-onset T1D died every year between the age of 25 and 40 years which is 20 times greater than the mortality of the USA general population (*JS Dorman 1984*). This excess is predominantly caused by acute complications (in the first decade of diabetes) and CAD (in the second and third decades).

Infectious diseases are also contributed to the premature deaths probably reflecting poor glycemic status in these subjects, however, data about long-term glycemic control and treatment regimes were not available in our study.

6.2. Differences in mortality between Havana and Allegheny County

This comparative study showed a dramatically higher mortality among people with childhood onset T1D in Havana, Cuba than in Allegheny County, USA. Type 1 diabetes is an uncommon disease in Cuba where incidence rates among children were 4-7 times lower than in the US (*Karvonen M 2000*), this may partly explain the much higher mortality in Havana.
We found further significant differences in the cause specific mortality patterns. More deaths in Allegheny County were attributed to acute complications than to any other cause, while in the Havana cohort the leading cause of death was nephropathy, which accounted for nearly half of the deaths. Interestingly the mortality rate for acute complications was lower in Havana than in Allegheny County (although this difference was not significant) while renal mortality was eight times higher in Havana than in the Allegheny County cohort. The lower acute complication mortality rate found in Havana might be due to the existence of universal care in Cuba that makes minimal care available to most of the patients. Ready access to acute care may reduce acute complications such as diabetic ketoacidosis which has been found as a major reason in deaths from acute complications in the USA. On the other hand the higher renal disease mortality may reflect the limited resources which prohibit adequate long-term care as that for advanced renal disease. In present-day Cuba, dialysis services are universally provided free of charge. However, there is no information regarding the availability of those services for patients with chronic renal disease in the past, during the study period. Unavailability of dialyses services were also likely to relate the high renal mortality in T1D in Japan seen in the early DERI reports (*Nishimura R 1996*). The DERI study reported that much of the premature mortality related to T1D was potentially preventable (*DERI Study Group Diabetes Care 1991*). It showed that the higher mortality rates in the Japanese cohort were basically due to a high proportion of deaths related to acute complications (42%) and kidney diseases (33%). Israel and Finland had lower mortality rates for both nephropathy and acute complications. The mortality related to nephropathy reported here was higher in the Cuban (405.1 per 100,000 person-year) cohort than in the DERI Japanese cohort (276.7 per 100,000 person-year). The low mortality rate attributed to cardiovascular diseases reported here may be due to the fact that the studied individuals are still relatively young.
A study using information from long-term follow-up of patients from the Joslin Clinic, showed that a combination of advances in diabetes therapy and in the control of infection brought substantial improvement in survival of people with diabetes (*Marks HH 1964*). Other

aspects related to long-term diabetes care such as the use of HbA1c (*Goldstein DE 1980*), blood glucose self-monitoring (*Consensus statement 1987*) and the use of preventive drugs such as angiotensin-converting-enzime inhibitors and angiotensin II receptor blockers in the prevention of diabetic nephropathy (*Lewis EJ 1993*) may have also played a role in the different mortality pattern showed here although they only became available toward the end of the observational period. While mortality in Allegheny County was clearly related to acute care (47% combining acute complications and infections), the Cuban mortality was related mainly to long term care (48% nephropathy). Similar results from Allegheny County have been described previously (*Tull ES 1996*). Mortality in the Havana city cohort was mainly related to long-term care and therefore linked to lack of resources such as availability of dialysis services or long-term glycemic control.

6.3. The effect of year of diagnosis and temporal trends in mortality

In an earlier report of the DERI study mortality, especially after 15 years duration of diabetes has declined during the last 30 years (*Nishimura R 2001*). The decline was most evident for the cases diagnosed in 1975–1979 compared with 1965–1969. SMRs at 20-year duration of diabetes showed a decreasing tendency over the study period, indicating that the improving prognosis of diabetic children was greater than that of the general population. The decline in mortality seemed to be similar in both Caucasians and African-Americans, although the prognosis of African-Americans with Type 1 diabetes was much worse than that of Caucasians after 25 years of diabetes. This has been revealed also by the combined DERI+EDC analysis. The timing of these improvements is consistent with the introduction of HbA1c testing, home blood glucose monitoring, and improved blood pressure therapy in the 1980s. The decline in mortality seen in the cohort diagnosed in 1975–1979 may reflect an improvement in prevention of long-term diabetic complications such as end-stage renal disease.

Our results showed furthermore an encouraging significant decline in the chronic complication mortality among Caucasians, which may reflect improvements both in the treatment and prevention of life-threatening, long-term diabetes complications such as end-stage renal disease and coronary heart disease. In our study, the decline in mortality for both complications was approaching significance (p=0.06). Similar borderline significant decline was seen in the mortality from chronic complications among African Americans. However, while acute complication mortality did not change much over time among Caucasians, there was a big improvement in Africans-Americans for those diagnosed in 1975-1979 compared to those diagnosed in 1965-1969.

In the Allegheny County-Havana comparative study improvements in survival have also been shown, however, it was not possible to expand the analysis to 1999 due to the lack of follow-up information in the Havana city cohort.

6.4. Racial and gender differences in mortality

Racial differences

The results of our analyses confirm those of prior reports using the Allegheny County Registry (*Nishimura R 2001, Dorman JS 1984*). In particular they support and extend the prior demonstrations of an excess total mortality in African-Americans after 20 years of duration (*Nishimura R 2001*) and 25 years maximum follow-up (*Tull ES 1996*). The current study has a maximum of 36-year follow-up and in addition to showing a four-fold difference between the African-Americans and Caucasians overall, also demonstrates the excess rate is largely related to acute complications.

One possible bias should be, however, mentioned regarding the identification of causes of death in the combined DERI+EDC study. Namely, that there was a difference in the availability of medical documents between African-Americans and Caucasians. The causes were decided by death certificates alone for only 14 percent of Caucasians but for 30 percent of African-Americans. It is possible therefore, that the classification may be less accurate in African-Americans and that we may have "overestimated" the deaths from acute complications. The truth is, however, that even if these "death certificate only" cases were excluded an excess would still be apparent.

Whether our findings are the results of a specific diabetes effect or of a more general racial disparity is difficult to determine, particularly as death from acute complications is likely to be the first manifestation of a general disadvantage. On the other hand, because the total mortality in the general population is also higher in African-Americans, the standard mortality ratios, as reported earlier for diabetes are relatively similar in both races (*Nishimura R 2001*). Thus, the poorer prognosis for African-Americans with Type 1 diabetes might reflect an underlying general, racial disparity in socio economic status, health care access and/or utilization and behavior. Unfortunately these data are not available for the majority of subjects in this study. The Allegheny County Registry has previously attempted to separate out any ethnic effect from social class in terms of the incident of Type 1 diabetes. However this was difficult for most African-Americans were in the lowest social class. Within this group of African-Americans the authors found little relationship between incidence of diabetes and socioeconomic status (*LaPorte RE 1981*). Socioeconomic status and availability of specialized care may not explain all of the present racial disparity in diabetes mortality rates and further exploration of possible culturally related factors would appear to be appropriate.

Although it has been proposed that some African-American individuals may have a different type of diabetes, namely phasic or "J" type, we think this is an unlikely explanation of our findings as this type of diabetes typically has an older onset than our subjects and is ketosis resistant (*Morrison EY 1995*).

The breakdown of death by race in the Allegheny County-Havana comparative study suggests that mortality in Allegheny County for African-Americans was worse than for Caucasians but similar to that seen in Havana for Hispanics. In cause-specific data by race, acute complication and infection related mortality clustered among African-Americans and this was responsible for most deaths among individuals in the Allegheny County cohort. While Hispanics had much higher mortality rate from nephropathy also when compared to African-Americans although in this case, confidence intervals are overlapped perhaps due to the low number of African-American cases. These racial differences can be explained mainly by the different health care systems of USA and Cuba (different access to acute and long-term care) and not likely by other specific race related factors (see section 6.2.).

Gender differences

The DERI study found that the male/female mortality ratio varied depending on where the person lived and the overall incidence; for example Finland and Allegheny County had higher mortality rates among men than among women while the opposite was evident for Japan and Israel (*DERI Am J Epidemiol 1991*). Though, we found lower mortality rates among males than females in the Allegheny County cohort and the opposite in the Havana City cohort, the differences were not significant. The World Health Organization Multinational Study of Vascular Disease in Diabetes reported an excess in male as compared to female mortality (2.50) in the Havana cohort of people with Type 1 diabetes (*Wang SL 1996*). In our study there was no gender differences found in SMR.

It is interesting to note that in the combined DERI+EDC cohort, the excess African-American mortality risk due to acute complications appears in both sexes, though it seems a little more marked in females. The reason for this is not clear, but it is consistent with our previous general findings (*Tull ES 1996*) and may relate to differences between males and females in risk factors for premature mortality from Type 1 diabetes (*Dorman JS 1984*). In this study, we also found that males were more likely to die from non-diabetes related causes than females. This is consistent with the literature, which demonstrates a greater number of accidents and suicides among young men than women (*DERI Study Group Diabetes Care 1991*). It should

also be noted that the Mortality Classification Committee has fully reviewed each death to determine the role of diabetes.

6.5. Incidences and predictors of CAD and nephropathy (MA, ON, ESRD)

Incidence of CAD and MA / ESRD

According to the results of DERI complication survey, the incidence of CAD increases dramatically after 25 years of diabetes duration reaching a cumulative incidence rate of 28% at 30 years. A decreasing incidence of diabetes complications, particularly overt nephropathy has been reported in the Type 1 diabetes population around the world over the past 15–20 years (*Hovind P 2003, Nordwall M 2004*). Mortality rates have also been decreasing (*Dorman JS 1984, Nishimura 2001*), most likely reflecting better management and the declining morbidity. Improved metabolic control, facilitated by the advent of self-monitoring of blood glucose and HbA1c testing and stimulated by the results of the Diabetes Control and Complications Trial (*DCCT 1993*), along with better blood pressure management (*Hovind P 2003, Nordwall M 2004*, *Dorman JS 1984, Nishimura 2001*) are likely to be major contributors.

Two recent prospective epidemiological studies, the Pittsburgh Epidemiology of Diabetes Complications (EDC) study (*Orchard TJ 2003*) and EURODIAB (*Soedamah-Muthu SS 2004*), a multicenter, clinic-based study in Europe, reported an incidence of total coronary events (including electrocardiogram [ECG] changes) of 16% over 10 years and 9% over 7 years, respectively, of follow-up in Type 1 diabetic patients. As the mean age at baseline was ~30 years, these incidence rates reflect the experience of those aged in their late 30s. In EDC, total CAD incidence (including angina and ischemic ECG changes) was >2% per year for those aged ≥35 years. A more recent report (*Pambianco G 2006*), using 12-year follow-up data, suggested annual major CAD event (myocardial infarction [MI], fatal CAD, or revascularization) rates of 0.98% for those with diabetes durations of 20–30 years (aged, on average, 28–38 years). These event rates were the same for both sexes, indicating a loss of the protection from CAD mortality that can be seen in females without diabetes experience. This is consistent with our findings in the DERI complication survey that the incidence rates are independent of gender and race.

We could not show any decline in the cumulative incidences of CAD in the DERI survey study which is supported by the EDC study (*Pambianco G 2006*) reporting no difference in the cumulative incidence of CAD by 20, 25, or 30 years' duration according to the year of diagnosis (1950 –1980). The benefits of improved diabetes care, therefore, do not (at least as

yet) appear to have reduced CAD morbidity, although a nice decline in CAD mortality by the year of diagnosis could be seen.

Our findings regarding the 5-year incidence/prevalence rate of early microalbuminuria is mostly consistent with previous data in the literature. Cross-sectional studies show 8-13% rates among children with a mean age of 14 years and 5-year duration of diabetes (*Salardi S 1990, Norgaard K 1989, Cook JJ 1990*). In one small longitudinal study with relatively low number of patients involved, Rudberg at al. found MA in 11% of the studied patients 7 years after the diabetes diagnosis (*Rudberg S 1993*).
Estimates of the prevalence of MA during childhood are confounded by the effects of puberty, as indicated by the documentation in several studies that MA is rare before puberty, even in individuals with diabetes of long duration (*Norgaard K 1989, Rudberg S 1993*). Furthermore, most studies of MA in children have been cross-sectional, with only a few being longitudinal (*Almdal T 1994, Rudberg S 1993*). When longitudinal studies have been undertaken, they have been clinic- rather than population-based, and the sample sizes have been relatively small (*Barkai L 1998, Jones CA 1998*). Determination of the prevalence of MA in relation to age, sex, duration of diabetes, glycemic control, and puberty and of the predictive value of MA in childhood was purposed in a large prospective population-based study (*Schultz CJ 1999*). The authors found a prevalence rate of 13% in a given geographic region in children with a median diabetes duration of five years (*Schultz CJ 1999).*

In our DERI complication survey data, the cumulative incidence rate of ESRD was 11.3 % at 25 years after onset. Klein et al. reported that cumulative incidence of renal insufficiency (serum creatinine >=2.0 mg/dl) and ESRD in a population-based cohort from Wisconsin was 14.4% at 10 years (*Klein R 1999*). In the EDC study based on children diagnosed with T1D at Children's Hospital of Pittsburgh between 1950 and 80, the cumulative incidence of renal failure at 25 years has declined from 21% to 9% for those diagnosed in the 1950's to the 70's, respectively. (*Orchard TJ 2002*). Differences in methodology, renal endpoints, definitions of ESRD and population characteristics make it difficult to compare these results. As few population based reports are available regarding the incidence of ESRD in T1D, international comparisons using cohorts diagnosed by the same criteria are thus of particular interest. Our previous report from the DERI study, which focused on the incidence of dialysis in Japan and Allegheny County, USA suggested that the rate in Allegheny County was lower than that in

Japan (*Matsushima M 1995*). Our observation period in the current study is now twice of that of the previous report, so further international comparison would be needed.

The EDC data suggests that natural history of Type 1 diabetes complications is generally improving, with major declines in total mortality and renal failure rates in individuals diagnosed after the mid-1960s. However, declines are not so impressive for overt nephropathy and any favorable patterns seen at 20 years have largely disappeared by 25 years' duration (*Pambianco G 2006*). In terms of temporal trends of ESRD, the current study indicates that a significant improvement has occurred especially between the cases diagnosed between 1965-69 and 1970-74. These results are consistent with a study in southeast Sweden indicating that cumulative incidence of persistent albuminuria declined among cases diagnosed between 1965 and 1980 (*Arnqvist HJ 1994*). An earlier decline in the incidence of diabetic renal disease reported for the cases diagnosed between 1930-50 (*Krolewski AS 1985, Andersen AR 1983*) was suggested to be due to improved metabolic control following the introduction of urine tests in 1950's, highly purified insulin in 1950's and blood glucose monitoring in 1970's. (*Kofoed-Enevoldsen A 1987*). The reasons for the current decline in the ESRD is likely to reflect the introduction of better anti-hypertensive therapy (*Goldstein DE 1980*), especially ACE inhibitors introduced in clinical practice in 1980's (*Lewis EJ 1993*), and better glycemic control following the introduction of HbA1c testing (*Lewis JB 1999*) and home blood sugar monitoring (*Consensus 1987*) in 1980's.

The dramatic decrease over time in the cumulative incidence of mortality and renal failure is consistent with two reports from the Allegheny County registry (*Nishimura R 2001*) and an Israeli mortality study (*Modan M 1991*). Despite the declining rate, however, a major excess mortality still exists in the Type 1 diabetes population compared with non-diabetic populations (*National Diabetes Data Group 1995*).

Our results from Allegheny County demonstrated a higher incidence of ESRD among African-Americans but an encouraging decline also in incidence for these diagnosed more recently. Similar findings have been reported in Type 2 diabetes (*Cowie CC 1989*). As a higher total mortality among African Americans with T1D have been reported in Allegheny County (*Nishimura R 2001, Tull ES 1996*), the role of ESRD in this disparity has not yet revealed.

Predictors of CAD and ON / MA

In our follow-up analysis based on the EDC study, traditional risk factors such as age and triglycerides were revealed as independent predictors of CAD while glycemic status and insulin resistance proved to be related to the development of nephropathy. Thus, our data supports previous suggestions that insulin resistance may play a role in the development of Type 1 diabetes complications, particularly overt nephropathy (*Erbey JR 1999, Stuhldreher WL 1992*). Fibrinolytic factors such as PAI-1 and its complexes with tPA did not show any direct effect on the development of CAD, however, strongly related to eGDR (marker of insulin resistance), independently of age, and thus are likely to be involved in the pathogenetic pathway.

An association between hypofibrinolysis and diabetes has previously been recognized (*Almer LO 1983, Fuller JH 1979*). It has been also shown from earlier studies that several well established cardiovascular risk factors such as smoking (*Meade TW 1979, Simpson AJ 1998*), hypertriglyceridemia (*Hamsten A 1985, Simpson AJ 1998*) and obesity (*Almer LO 1975*) are also associated with a decrease in fibrinolytic activity. In particular the association with triglycerides are relevant to the current analyses, as both fibrinolytic variables were significantly correlated with triglycerides which was the major independent multivariate predictor of CAD. It is thus likely that if triglycerides contribute above and beyond their association with fibrinolysis (as seems likely) that they would be a stronger statistical predictor, even though some of that prediction may represent altered fibrinolysis.

Since deep assessment and analysis of potential traditional and novel predictors for CAD and ON was not the main purpose of our current study, it has limitations addressing this very complex issue, although results are consistent with other EDC analysis employed an extensive array of risk factors and involved stored plasma/serum samples acquired over the 10-year follow-up. In this study the results suggest that glycaemic exposure, insulin resistance, and possibly oxidative damage may be important 'initiators', while blood pressure and lipids have a greater role as accelerators later in the process (*Yishak AA 2006*). These results are consistence with our findings in a different substudy of the same cohort which was focusing on the fibrinolytic changes in T1D. Our earlier observations (*Orchard TJ Kid Intl 2002*) showed similar effects using a different temporal analysis, i.e. eGDR and HbA_1 predict overt nephropathy over the long term (5–10 years post-measurement), while blood pressure and lipids predict in the short term (0–5 years post-measurement). Furthermore, though fibrinogen,

pulse and eGDR were significant univariate predictors of overt nephropathy, these did not appear to contribute independently of log AER, log IgG and LDL particle size.

Urinary albuminuria (AER) was proved to be a strong independent predictor of overt nephropathy in agreement with earlier reports (*Mogensen CE 1984*). A deleterious influence of serum total or LDL cholesterol on renal function decline and/or progression of albuminuria has been previously reported among individuals with Type 1 diabetes. It seems likely that it is mainly the small dense LDL particles that are related to incidence of overt nephropathy, as it has also been reported for CAD in this cohort (*Soedamah-Muthu SS 2003*). Interestingly, this latter analysis showed HDL subclasses 4 and 5 to be independently (negatively) related to CAD, while a positive correlation was observed for H3 subclass. No such associations were seen in this analysis for overt nephropathy. In a similar nested case–control study from the EURODIAB cohort by Chaturvedi (*Chaturvedi N 2002*), elevated cholesterol, triglycerides, LDL, Apo B and diminished LDL particle size were associated with albuminuria, although LDL subclasses were not measured.

Some similarities can be observed with CAD predictors (e.g. LDL particle size), whereas other factors predictive of CAD in this cohort (e.g. VLDL subclasses, hypertension) do not seem to be as strongly related to overt nephropathy. Nonetheless, the results are consistent with the hypothesis that similar risk factors may accelerate disease in both the arterial blood vessels (atherosclerosis) and glomerulus (glomerulosclerosis), when they are damaged by initial oxidation (particularly the former) and/or glycosylation (particularly the latter).

Several factors can influence the development of early microalbuminuria in children with Type 1 diabetes. According to Schultz at al. the cumulative probability of developing MA during childhood is related to sex, HbA1c, duration of diabetes, and pubertal status. MA is rare before puberty except in those who were 5 years of age at diagnosis of diabetes. Although these factors affect MA prevalence in childhood, the cumulative probability is similar irrespective of age at diagnosis, indicating the importance of glycemic control and diabetes duration in the pre-pubertal years (*Schultz CJ 1999*).

In contrary, in our study examining 5 and 10-year follow-up results of patients with childhood (<18 years) onset Type 1 diabetes, we found no effect of glycemic control and other traditional risk factors on the development of early microalbuminuria. This discrepancy might be probably explained by the generally high baseline HbA1c values and the relatively low number of subjects involved. Our main result, however, supports earlier findings that 5-year incidence of MA is significantly lower among patients with early childhood (0-4 years) onset

T1D compared to those in higher age groups (5-9, 10-17 yrs) by diabetes onset. Biologic maturity measured by the Tanner-score determines strongly the progression to MA confirming the results from large studies that hormonal changes in puberty can accelerate the development of microalbuminuria in children with diabetes.

6.6. Survival after CAD–revascularization and ESRD–renal replacement therapy

According to our results the incidence of non-fatal myocardial infarction and revascularization therapy increased dramatically after two and a half decades of diabetes duration, however, probably due to the benefits of RV and other conservative-preventive therapies in those who developed CAD, the incidence of fatal MI remained relatively stable.

The BARI (Bypass Angioplasty Revascularization Investigation) randomized trial was designed to test whether percutaneous transluminal coronary balloon angioplasty (PTCA) compromised 5-year survival compared with coronary artery bypass grafting (CABG) in patients with multivessel coronary artery disease. After 5 years of follow-up, overall survival was similar for the two revascularization strategies (*BARI Investigators 1996*); however, after 7 years of follow-up, CABG survival was statistically superior (*BARI Investigators 2000*). An unexpected finding of the BARI trial was that among patients without treated diabetes, survival rates for the PTCA and CABG randomized groups were almost identical throughout the 7 years of follow-up, whereas among patients with treated diabetes, the CABG group had significantly better survival. The survival difference was attributable to reduced cardiac mortality (*BARI Investigators 1997*). This result was confirmed also by the final – 10-year follow-up – data of the BARI cohort.

In patients with Type 2 diabetes, CABG conferred a consistent, clinically relevant, absolute survival benefit over balloon angioplasty that diminished somewhat over extended follow-up because patients in both groups had higher event rates. Five-year mortality results from Arterial Revascularization Therapies Study (ARTS) - diabetes: 13.4% stents vs. 8.3% CABG, relative risk =1.61, p=0.27; no diabetes: 6.8% stents vs. 7.5% CABG, relative risk=0.91, p=0.71 - (*Abizaid A 2001*) support the finding that CABG may have particular advantages for patients with diabetes. It remains to be seen whether advances in percutaneous procedures and medical management over the past decade will make contemporary angioplasty a reasonable option in this cohort. The steady incidence of cardiac events over the 10 years of follow-up in both treatment groups in the BARI study emphasizes that coronary revascularization does not reverse the underlying pathophysiology of coronary disease.

In our results from the DERI survey both forms of revascularization therapy seemed to be beneficial even in patients without myocardial infarction thus providing support for the early revascularization of Type 1 diabetes individuals with CAD. There was no significant difference found between the revascularization strategies probably due to the considerably

low number of CAD deaths and events. Further limitation of the study is that it was based on self-reported data (*Appendix 1.*) where exact definition of myocardial infarction could not be made and no other relevant factors (glycemic control, smoking, lipid levels and other risk factors) were collected.

The introduction of coronary stents and other technical refinements in angioplasty and surgery did not alter the conclusion from these results. Although some might argue that these procedural refinements make the results obsolete, we believe that our observations with respect to death and MI remain applicable to contemporary practice. Overviews of the randomized trials of bare metal stents (*Brophy JM 2003, Kandzari DE 2006*) and drug-eluting stents (*Babapulle MN 2004, Tsimikas S 2006*) show that these devices have no significant advantage regarding mortality or MI compared with balloon angioplasty despite striking reductions in rates of restenosis and repeat revascularization procedures. Furthermore, observational studies of patients undergoing angioplasty indicate that restenosis does not confer a worse prognosis concerning survival (*Weintraub WS 1993*). Underuse of evidence-based medical therapies is unfortunately common among patients with coronary disease. Clinical outcomes for all patients may be improved further by providing long-term aggressive medical management after revascularization.

As a result of our analysis in the Allegheny County cohort (DERI study), the cumulative survival after end-stage renal disease (ESRD) improved by the year of diabetes diagnosis probably reflecting better access and care in patients with renal disease.

Approximately half of the subjects survived 10 years after the introduction of renal replacement therapy (RRT) in the current study. The declining tendency in mortality of ESRD did not reach statistical significance, probably because of the small number of events. It thus is critical that a longer observation period be examined in the future.

Cowie et al reported that mean survival of African-Americans after RRT was shorter than Caucasian (*Cowie CC 1994*), however, in this study, little difference was observed, and in multivariate analyses race did not affect the prognosis. Though, the incidence rate of ESRD in African-Americans was significantly higher than that of Caucasians, once they had ESRD, the risk of death was not greatly affected by race.

In the 1970's and 1980's, many reports indicated that renal transplantation offered better survival than dialysis (*Khauli RB 1986, Parfrey PS 1985*). According to United States Renal Data System (USRDS) coordination center (*USRDS 2001*), expected survival of transplant recipients was more than twice that of dialysis recipients in the same age range, and the 5-year

survival after dialysis alone, transplantation from cadaveric donor and transplantation from living donor were 29.2%, 75.2% and 83.0%, respectively in diabetes patients (*USRDS 2001*). In the current study, the transplant recipients also had a better survival than patients on dialysis, and the 5-year survival after dialysis alone (32.8%) and transplantation (79.6%) observed in this study were similar to USRDS. Part of the better survival is likely to be explained by the fact that healthier patients are placed on the waiting list for transplantation, so that long-term survival is better among those on the waiting list who eventually undergo transplantation (*Wolfe RA 1999*). In a report by Wolfe et al., the relative risk of death following cadaveric transplantation compared to dialysis awaiting transplantation was as low as 0.18 in the 20-39-year age group with diabetes (*Wolfe RA 1999*). The absolute risk was not comparable with this study, as the methodology was different, however, the relative risk (0.25) was similar.

Individuals treated with pancreatic-kidney transplantation have also been reported to have a better survival than those undergoing kidney transplantation alone (*Becker BN 2000, Smets YF 1999*). In our current study, pancreatic-kidney transplantation recipients tended to have better survival than kidney transplant recipients. The difference, though large, did not reach statistical significance in life table analysis and Cox time-dependent analyses as the number of the events were relatively small, reflecting the more recent introduction of pancreatic-kidney transplantation. Longer follow-up will be necessary to evaluate this issue in this cohort.

One of the limitations of our study is that we could not ascertain end-stage renal complication status for 15 percent of the cases whose living status was confirmed, due mainly to participant reluctance or that of their relatives, including parents, to allow us to contact subjects. Although survival after RRT was higher with transplantation compared to dialysis alone, these data do not permit a full evaluation as we had no details concerning the clinical state of subjects at the onset of RRT. It is likely that those with more severe co-morbidities were not transplants candidates.

7. Summary and conclusion

The introduction of insulin into clinical use in the 1920s dramatically changed the life of children with Type 1 diabetes. Although much of progression has so far been achieved in their life-expectancy and life quality, health outcome indicators are still proved to be worse in Type 1 diabetes patients compared to non-diabetic population. Individuals who develop diabetes remain at substantially greater risk of early mortality in which the major microvascular and macrovascular complications of diabetes play an essential role.

The presented studies are expected to give epidemiological answers to the number of issues concerning childhood-onset Type 1 diabetes mortality, its major complications and the link between them. My theses below are based upon these major findings.

7.1. Theses

a) Beyond the unexpectedly common acute complications, coronary artery disease as a macrovascular and renal disease as a microvascular chronic complication attributed significantly to premature deaths of patients with Type 1 diabetes.

b) Racial differences in cause-specific mortality from Type 1 diabetes have been revealed. The excess mortality among African-American diabetes patients is particularly linked to acute diabetes complications, mainly diabetic ketoacidosis, perhaps reflecting poor health care access or utilization. In Hispanics, however, the leading cause of death seems to be nephropathy which may reflect limited resources for those with advanced renal disease. While mortality in the US was clearly related to acute care, excess mortality in Cuban patients was mainly attributable to long-term care reflecting the effects of two largely different health care systems on mortality and outcomes. This can lead us to the conclusion that different strategies might be needed to improve survival among individuals with Type 1 diabetes in USA and Cuba.

c) An encouraging improvement has been achieved overall in the 20-year mortality rates for all major causes (especially chronic diabetes complications among Caucasians) by calendar year of diagnosis probably due to the better preventive strategies of the recent years, though African-Americans are still more likely to be affected by diabetes-related deaths. Since acute complications of diabetes may be the most amenable to intervention, a focus on their prevention should be a high priority.

d) Incidence of overt nephropathy has been nicely decreasing in recent years, however, the benefits of improved diabetes care, at least as yet, do not appear to have reduced morbidity of coronary artery disease, although a nice decline in their mortality by the year of diagnosis can be seen. This might also be explained by different predictors affecting the development of renal disease and coronary artery disease, as glycemia itself can more directly contribute to the former one while insulin resistance rather play a big role in the latter complication.

e) The development of early microalbuminuria might be considerable in children within five years of diabetes duration. Age at onset and pubertal maturity largely contribute to the risk of microalbuminuria. Although HbA1c might be less related to the early development of microalbuminuria, poor glycemic status may have long-term consequences for the risk of subsequent nephropathy and for cardiovascular risk in postpubertal children.

f) Once complication developed, right tertiary prevention strategy should be planned to improve life quality and prolong life-years. Both forms of revascularization therapy (i.e. coronary artery bypass grafting and percutaneous transluminary coronary angioplasty) might be beneficial for patients with Type 1 diabetes who underwent myocardial infarction. Our data also provide support for early revascularization as a secondary prevention strategy showing better survival in those who undergo revascularization before the irreversible myocardial event. The incidence of end-stage renal disease requiring some form of renal replacement therapy shows an encouraging decline for those patients diagnosed more recently. Survival after renal replacement therapy is greater with transplantation compared with dialysis therapy alone. Combined pancreatic-kidney transplantation seems to be beneficial over renal transplantation alone.

7.2. Conclusive remarks

These epidemiological data might help diabetes specialists in order to use appropriate prevention strategies in their everyday clinical practice and diabetes care, nevertheless they might contribute to a better understanding in the natural history of the disease.

The challenge to society is to deliver intensive diabetes therapy to Type 1 diabetic patients at large, in a cost-effective manner, by teams with appropriate expertise. Our data suggest optimism toward the way that adequate diabetes care will become available to all children and adolescents who developed the disease, thus they achieve life expectancy and life-quality same as in the general population.

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10. Appendices

10.1. DERI Study complication survey

Diabetes Update

Date _____/_____/____ Family ID _____Case ID _____

Name _____

Last First Maiden

Please correct your name , address and phone number if the information below is wrong.

Address

Phone

Please answer the questions below and fill in the year and month of the first diagnosis where appropriate.

1) Have you ever had Renal Dialysis?

☐ **no** ☐ **yes (start date: year / month)**

2) Have you received Renal Transplantation?

☐ **no** ☐ **yes (first surgery: year / month)**

If yes, which one have you received?

☐ **renal only** ☐ **both renal and pancreas**

3) Have you received Photocoagulation Therapy (laser treatment of the eyes)?

☐ **no** ☐ **yes (first therapy: year / month)**

4) Do you have history of Myocardial Infarction (Heart Attack)?

☐ **no** ☐ **yes (first attack: year / month)**

5) Have you ever had Angioplasty (PTCA) ?

☐ **no** ☐ **yes (first therapy: year / month)**

6) Have you ever had Coronary Bypass Surgery?

☐ **no** ☐ **yes (first surgery: year / month)**

7) Do you have history of Stroke?

☐ **no** ☐ **yes (first stroke: year / month)**

8) Have you ever had an Amputation?

☐ **no** ☐ **yes (first surgery: year / month /site:)**

9) Have you ever had an ilio femoral bypass surgery (bypass of the artery to the legs)?

☐ **no** ☐ **yes (first surgery: year / month)**

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