Incidence Trends and Environmental Determinants of Type 1 Diabetes in Lithuania and Sweden

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2003
To my family – in Sweden and Lithuania
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ABSTRACT

Aims: Variation of diabetes incidence over time in countries with different incidence levels and socio-economic conditions, and in an age span beyond the childhood years, may give clues for it’s causes. The aim of the present studies was to compare the time trend in Lithuania and Sweden, as well as incidence level in urban and rural areas in Lithuania, that differ with respect of wealth distribution. Incidence change over time was analysed in a broader age span in Sweden to check whether the increasing incidence of childhood diabetes may be explained by a shift to an earlier age at diagnosis. Associations between certain environmental factors and the risk of diabetes in childhood were also investigated.

Materials and methods: I-III. Data from nation-wide prospective type 1 diabetes registers in Sweden and Lithuania, covering the populations of children (0-14 years) and young adults (15-34 and 15-39 years, respectively) during different time periods between 1983 and 2000. IV. Case-control study using information about infections and breast-feeding prospectively recorded in health care booklets (117 cases; 270 controls from Lithuania). V. Case-control study using detailed interviews on dietary habits one year before diabetes diagnosis, and routinely recorded growth data (99 cases; 180 controls from Stockholm region).

Results: Between 1983 and 2000 the average incidence in 0-14-year age group was 28.9 and 7.5/100,000/year in Sweden and Lithuania, respectively. The largest difference between the two countries in the 0-34-year group was found among the youngest children, and the difference decreased with age.

Incidence increased significantly in both countries in the 0-14-year group by an average 2.2% per year in Sweden and 2.3% in Lithuania from 1983 to 2000. In Sweden the increase occurred in all age groups and more so in the younger ones, while in Lithuania a significant increase was found only in the 10-14-yr group.

The incidence of type 1 diabetes in the 0-34-year group in Sweden did not increase from 1983 to 1998. Comparison of four 4-yr periods showed a clear increase over time among 0-14-year old children, but a tendency of a decrease in the older age groups, especially in men, thus indicating a shift towards an earlier age at diagnosis.

The incidence of type 1 diabetes in the 0-39-year group in Lithuania was lower in rural areas than in towns and cities (7.1, 9.0 and 8.8/100,000/year,
p<0.001). The differences were most marked among children aged 0-9 years. Between 1991-1995 and 1996-2000 the overall incidence increased for both 0-39-year old Lithuanian males and females, but the pattern of change differed between the sexes, by the urban-rural setting and age group.

Exposure to one or more non-specific infection during the first half year of life was associated with reduced diabetes risk among 0-14-year old Lithuanian children (OR=0.60; 95% CI 0.37-0.98, adjusted for confounders). The association was stronger in the 5-14-year group (OR=0.47; 95% CI 0.26-0.87).

Higher weight-for-age and higher energy intake were independently associated with increased risk of type 1 diabetes: odds ratios (95% CI) for medium and high levels of energy intake were 1.33 (0.52-3.42) and 5.23 (1.67-16.38), and for weight-for-age 3.20 (1.30-7.88) and 3.09 (1.16-8.22), respectively. High intake of carbohydrates, disaccharides and sucrose in particular, were significantly associated with type 1 diabetes risk independently of high intake of energy.

Conclusions: Taken together, our findings suggest, that environmental factors associated with socio-economic conditions and wealth, perhaps particularly during the early childhood, may be important for the occurrence of type 1 diabetes. This possibility is also supported by the results of the case-control studies, as exposure to infections, nutrition habits and growth pattern are known to differ depending on the socio-economic conditions in childhood. Life style habits leading to less exposure to microbial antigens early in life, and to a high intake of energy and higher growth rate in childhood may contribute to the increase of childhood-onset type 1 diabetes.

Keywords: type 1 diabetes mellitus, epidemiology, childhood, incidence, secular trend, case control study, risk factors, infections, energy, nutrients, weight, height.
This thesis is based on the following publications, which will be referred to by their Roman numerals:


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INTRODUCTION

The epidemiological method

Epidemiology is a scientific method aimed at studying the causes of disease at the population level (1, 2). Epidemiological studies of type 1 diabetes are based on well-ascertained registers that have been established throughout the world during the last two decades (3-5). Descriptive studies analyse the variation of incidence over time and geography, and may help yield new hypotheses on the aetiology of diabetes. Changes in incidence, which may be described as age, calendar period or birth cohort effects, may be expected to reflect the distribution of environmental risk factors in a population. Ecological studies measure the association between a risk factor and the risk of disease on the population level – over geographical areas or time. They may also give clues for aetiological hypotheses, but provide weak evidence for causation, as they do not reflect exposures at an individual level. Hypotheses about risk factors may be further explored in analytical epidemiological studies and animal models for understanding the underlying mechanisms. Analytical studies (population-based cohort or case-control) analyse at an individual level the associations between certain exposures and the risk of developing a disease.

Diabetes mellitus – diagnosis and classification

Definition

The term diabetes mellitus describes a metabolic disorder of different aetiology characterized by chronic hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (6).

Diagnosis

Increased thirst and urine volume, unexplained weight loss, recurrent infections and, in severe cases, drowsiness and coma are the symptoms that prompt the clinical diagnosis of diabetes. According to the diagnostic criteria of the World Health Organisation (WHO) (7), a casual (random) glucose measurement of 11.1 mmol/l or above in plasma of venous blood (≥10.0 mmol/l in whole venous blood) establishes the diagnosis of diabetes. When casual glucose values fall in the uncertain range (between 5.5 and 11.1 mmol/l in plasma of venous blood), an oral glucose tolerance test (OGTT) is performed for the establishment of diagnosis. Diabetes is diagnosed if a
fasting blood glucose value of $\geq 7.8$ and/or $\geq 11.1$ mmol/l two hours after glucose load is measured in the plasma of venous blood.

*Diabetes in children.* Diabetes in children usually presents with severe symptoms, very high blood glucose levels, marked glycosuria, and ketonuria. In most children the diagnosis is confirmed by blood glucose measurement and OGTT is neither necessary nor appropriate for the diagnosis (6, 7). Only a small proportion of children and adolescents may present with less severe clinical symptoms and require a measurement of fasting blood glucose and/or an OGTT for diagnosis.

**Classification**

In 1980 the WHO Expert Committee proposed two major clinical classes of diabetes mellitus and named them insulin-dependent (IDDM) or type 1, and non-insulin-dependent (NIDDM) or type 2 (8). The 1985 revision of this classification became an international standard used in both clinical practice and research. The revision retained the clinically descriptive classes of IDDM and NIDDM, but omitted the terms type 1 and type 2 diabetes, that were to be used only as synonyms of IDDM and NIDDM (7). The basis for the distinction between the major subclasses of diabetes mellitus was the patient’s dependence for survival on insulin, judged to be present when the “classical” symptoms of diabetes were associated with greatly raised concentrations of glucose and ketone bodies in the blood and urine. Other clinical classes of diabetes mellitus included malnutrition-related diabetes, other types of diabetes associated with certain conditions and syndromes, impaired glucose tolerance and gestational diabetes mellitus.

*Proposal of a new classification.* In 1997 the American Diabetes Association (9) and in 1998 the WHO working group (6) proposed a new classification, including clinical stages and aetiological types of diabetes mellitus and other categories of hyperglycaemia. Clinical staging reflects the varying degree of hyperglycaemia during the natural history of diabetes progression, and aetiological classification designates defects, disorders or processes that often result in diabetes mellitus (the terms type 1 and Type 2 diabetes were retained). However, although more correct from the theoretical point of view, the new aetiological classification would not currently be feasible at a wide international basis, as it requires auto-antibody determinations that can only be performed in certain centres. The diagnostic value of the fasting plasma glucose concentration was lowered to 7.0 mmol/l (to 6.1 mmol/l for the whole blood). A category of impaired fasting glycaemia (IFG) was introduced, with fasting plasma glucose values within the interval $\geq 6.1$ and $< 7.0$ mmol/l ($\geq 5.6$ and $< 6.1$ mmol/l in whole blood).
Descriptive epidemiology of type 1 diabetes

*Diabetes in childhood (age group 0-14 years)*

Type 1 diabetes diagnosed below the age of 15 years shows a prominent worldwide variation of incidence rates both between and within the continents and even in closely geographically related areas (3, 4). Between the continents the lowest incidence rates are found in Asia and South America and the highest in Europe. Incidence rates registered during 1990-1994 among 100 populations worldwide differed >350-fold: from 0.1 per 100,000 in China and Venezuela to 36.8 and 36.5 per 100,000 in Sardinia and Finland (4). The largest intercontinental variation of incidence appears among European children, varying from 3.6 per 100,000 in Macedonia to 43.9 per 100,000 in two regions in Finland (10). Overall, the incidence rates are high in Northern and North Western Europe and low in Central, Eastern and Southern Europe, with the exception of Sardinia where the incidence is much higher than in any neighbouring country (10, 11).

A marked variation of incidence is also seen within a relatively small geographical area, among the countries in the region of the Baltic Sea (Finland, Estonia, Latvia, Lithuania and Poland). The average age-standardised yearly incidence of type 1 diabetes per 100,000 during the period 1983-1988 ranged from 6.0 in Poland to 36.9 in Finland for boys (6.4 and 31.6 for girls, respectively) (12). In Sweden the incidence was 25 per 100,000 over a 15-year period between 1978 and 1992 (13), being the third highest reported in Europe and the fourth highest worldwide (3). While in Lithuania the incidence of type 1 diabetes is markedly lower compared to Sweden and to the European average – between 1983 and 1992 the average incidence rate was 7.1 per 100,000 (14).

Significant geographical variation is found also within genetically homogeneous populations: Sweden (15, 16), Finland (17), Norway (18, 19), as well as Germany (20) and Austria (21). In other countries incidence varies depending on the urban-rural setting, often expressed as population density, although the association is not consistent (22-26).

The worldwide geographical variation in incidence probably reflects the global distribution of major ethnic populations with a different degree of genetic susceptibility. However, the variation of incidence within the same ethnic group, and even in the same country, suggest that environmental factors are important in the aetiology of type 1 diabetes.
**Variation by age**

In most populations incidence varies according to the age at diagnosis: incidence rates are lowest in the 0-4 and highest in the 10-14-year age group (5, 12, 13). Studies including age groups beyond 14 years showed, that for both boys and girls incidence peaks around the puberty, decreases thereafter, and seems to plateau after the age of 20 years (16, 19, 27, 28).

**Seasonal variation**

In most countries throughout the world a seasonal variation in the month of diagnosis of type 1 diabetes is observed, so that a lower incidence is consistently registered during the warm summer months compared to cold or cooler winter months, especially in countries where the seasons are well defined (3). A statistically significant heterogeneity in the seasonal distribution is present among 16 regions participating in the EURODIAB ACE framework, all of them showing peaks of type 1 diabetes incidence in winter or early spring (December through March), the pattern being similar for each of the three age groups and for both sexes (29). Such observations also point to some environmental influences, resulting in a similar seasonal pattern of the disease onset.

The incidence of type 1 diabetes is negatively associated with average yearly temperature (30) and in Europe it is positively associated with latitude (31). However, the very high incidence in Sardinia and relatively low incidence in Iceland shows that the north-south gradient has exceptions. Also within one country climatological factors are found to influence the risk of type 1 diabetes. When Sweden is divided into 11 regions according to north-south gradient, a significant effect of latitude and season at onset is found (16). Both mean sunshine hours and mean monthly temperature measured in each county in Sweden are also independently negatively associated with incidence rate of type 1 diabetes (13), which may partly explain the north-south gradient and seasonal variation of the disease. Since blood glucose (32) and insulin levels (33) are higher during winter period, both the north-south gradient and the seasonal pattern of variability might be explained by cold, which could unmask the insulin deficiency and thus precipitate the clinical onset of type 1 diabetes. Lower levels of vitamin D during the winter may be an alternative explanation (34, 35).

**Incidence change over time**

The increase in the incidence of childhood type 1 diabetes over time seems to be a global phenomenon (4). In Europe, the incidence of diabetes increased by 3.2% per year on average from 1989 to 1998 in children under 15 years of age, and by 4.8% in those under 5 years of age (10). The age at diagnosis of
type 1 diabetes has been moving towards younger age since 1965 among the Finnish children aged 1-14 years: in the 1970s the incidence was steepest in 5-9 year old and since the mid-1980s in those younger than five years at diagnosis, so that the differences in incidence between the age groups have almost disappeared (36, 37). A recent comparison of the time trends in Sweden also showed that the increase over time was most rapid in the 0-4-year age group, and indicated a shift towards a younger age at diagnosis in children below 15 years of age (38). From 1983 to 1992, a tendency toward an increase of 1.4% per year was also found in Lithuania (p>0.05) (14). More recent reports have shown that during the 1990s the incidence of childhood diabetes increased in the three Baltic countries as a whole (10, 39). However, the tendency toward an increase among Lithuanian children was not statistically significant (39).

The temporal changes of incidence are too rapid to be explained by an increase in the pool of genetically susceptible individuals in the population, or by a change in genetic susceptibility, thus they should be caused by a change in the environmental exposures. It might be argued, that type 1 diabetes is not becoming more common but that due to changes in the environment susceptible individuals develop clinically overt disease at an earlier age.

**Diabetes in adults (age group >14 years)**

The epidemiology of type 1 diabetes beyond 14 years-of-age is less extensively studied due to more difficult case ascertainment and problems with classification (40), although a large proportion of type 1 diabetes cases are diagnosed later in life (16, 41-43). The available data suggest that incidence levels in different countries vary less in the 15-29-year, compared to the 0-14-year age group Figure 1 (16, 19, 22, 24, 27, 28, 44-51). In the adult age groups a male preponderance is found in most populations.

Very few studies have analysed the time trend of type 1 diabetes incidence in the older age groups. A tendency of an increase was found in both children (0-14 y) and young adults (15-29 y) in Turin (Italy) 1984 through 1996 (52). In West Yorkshire, the incidence tended to increase in the 0-14, and was stable in the 15-29 years age group between 1991 and 1999 (53). However, in Belgium (28) the change of incidence over time seemed to have opposite directions for childhood-onset and adult-onset type 1 diabetes, supporting a shift towards an earlier age at diagnosis rather than an overall increase in incidence.
Figure 1. Incidence of type 1 diabetes per 100,000 per year in age groups 0-14 and 15-29 years by sex (16, 19, 22, 24, 27, 28, 44-51)
Aetiology and pathogenesis of type 1 diabetes

Type 1 (insulin-dependent) diabetes is an autoimmune disease caused by the selective destruction of the insulin-producing pancreatic beta cells (54). The cause of the beta cell destruction was not understood until mid-1970s when islet cell antibodies (ICAs) were first found in sera from type 1 diabetic patients with polyendocrine disease (55). Since then an accumulating amount of evidence has suggested that type 1 diabetes results from autoimmune destruction of the insulin-producing pancreatic beta-cells (54, 56, 57). During the 1980s and early 1990s several other islet autoantigens were identified. The main autoantigens recognized by ICA are glutamic acid decarboxylase (GAD65) and protein thyrosine phosphatase-like molecule (IA-2 or ICA512) (58). The third major autoantigen, not recognized in the ICA test, is insulin (IAA) (59, 60).

The available evidence suggests that cellular autoimmunity is responsible for the destruction of the beta cells, and is primarily mediated by cytotoxic T-cells reactive to islet antigens (56). Recent evidence points to apoptosis as the main form of beta-cell death in animal models of type 1 diabetes mellitus, and probably also in humans (61). The autoantibodies probably do not play a major role in the destruction of the beta cells, still they are markers of the ongoing beta-cell damage during the pre-clinical period, and are the most thoroughly characterized immune phenomena associated with type 1 diabetes in humans.

Pathological findings in the pancreas

Data obtained from autopsies of diabetic patients who died shortly after the clinical diagnosis of type 1 diabetes show that the key pathological features in the pancreas are mononuclear cell infiltration of the pancreatic islets, called insulitis, and near total selective reduction of the insulin-producing beta cells (62). It is estimated that at the time when clinical symptoms appear, 60-80% of beta cells are destroyed (57). In humans the knowledge about the nature of the insulitis lesion during the pre-diabetic period and at the time of clinical presentation is limited because of the risks associated with pancreatic biopsy. However, some new reports show that pancreatic biopsy under laparoscope is a relatively safe procedure without serious complications (63). In the biopsy material the majority of recent-onset adult type 1 diabetic patients had a T-cell-predominant infiltration of islets and hyperexpression of major histocompatibility complex class I antigens on islet cells, considered as evidence of cellular autoimmunity (63). Moreover, the presence of insulitis was closely associated with autoantibodies to GAD or IA-2. The inflammatory infiltrate of insulitis contained CD8+ and CD4+ T-cells,
macrophages and B cells and some natural killer cells, where the CD8+ T-cells and macrophages were predominant (64, 65).

**Genetic predisposition**

It is well established that genetic predisposition is involved into the aetiology of type 1 diabetes (66, 67). In humans the HLA (human leukocyte antigen) region on the short arm of chromosome six contains genes of the major histocompatibility complex (MHC), which are highly associated with the risk of type 1 diabetes (68). The associations are complex, as several different HLA genes are to a variable degree associated with susceptibility or resistance to the disease. However, the evidence available suggests the primary involvement of genes in the HLA class II region, encoding DQ molecules. It seems that the degree of genetic predisposition for type 1 diabetes carried by an individual results from a particular combination of his or her DQ molecules, conferring a varying degree of susceptibility or resistance (69), whereas DQ molecules which determine resistance are dominant over those determining susceptibility (70). Other genes in the HLA complex or some non-HLA genes might also be involved into the pathogenesis of type 1 diabetes. A genome-wide search for type 1 diabetes susceptibility genes found eighteen different chromosome regions associated with the disease, besides the insulin gene on chromosome eleven (71) and major histocompatibility complex on chromosome six, however except the latter the associations were weak (72). Although more prevalent in type 1 diabetes patients compared to controls, none of the putative susceptibility alleles are unique to the patients, indicating that the role of genetic determinants is not causal but rather permissive (73).

**Indications for the role of environment**

The pattern of familial transmission and differences in the distribution of genetic determinants among people with type 1 diabetes, as well as in the background population in different ethnic groups (74, 75) indicate that the determinism is polygenic and multifactorial (56, 73). The prevalence of pre-clinical diabetes (positivity for islet auto-antibodies) among healthy schoolchildren and first-degree relatives is higher than the expected prevalence of overt diabetes, suggesting that some pre-diabetic subjects will never progress to clinically overt diabetes (76, 77). Comparison of the concordance in monozygotic and dizygotic twins provides information regarding the relative importance of genetic and environmental factors. A population-based study of twin pairs in Finland found only 23-27% concordance rate of type 1 diabetes for monozygotic and 5-4% for dizygotic twins (78, 79), which points to a strong contribution of non-genetic factors as well as to a polygenetic mode of inheritance. Rapid incidence changes over
time, seasonal variation of the clinical onset, as well as incidence differences in close geographical areas and even in the same country also suggest that environmental factors are important in the aetiology of type 1 diabetes.

The natural history of type 1 diabetes
Follow-up studies of identical twins (80, 81) and other first-degree relatives of people with type 1 diabetes (82) have shown that the pre-diabetic phase of type 1 diabetes lasts several years. Several studies in Germany (83), Finland (84) and USA (Colorado) (85) are currently prospectively following from birth first-degree relatives of patients with type 1 diabetes, and individuals at increased genetic risk from the general population to measure the expression of autoantibodies and the development of type 1 diabetes. All of these studies show that anti-islet antibodies commonly develop between the age of 9 months and 3 years, although in some children they can appear even earlier or later. Insulin autoantibodies often develop as the first (but are transient most often), with ICA or GAD being the second most frequent. Most individuals progressing to clinical diabetes have multiple (two or more) anti-islet autoantibodies by the time of clinical presentation. However, even among the children with similar HLA-associated genetic risk, the time from the first appearance of the autoantibodies to the diagnosis of diabetes varies from one to several years (86).

Regardless of its aetiology, diabetes progresses from normal to impaired glucose tolerance (and/or impaired fasting glucose) and further to clinical diabetes, which may not require insulin for treatment, may require insulin to achieve adequate metabolic control, and require insulin for survival (6). During the process of diabetes development individual subjects may move from one clinical stage to another in either direction.

Environmental factors associated with type 1 diabetes
Environmental factors are believed to initiate and modify the pathogenic process of beta-cell destruction: depending on the timing and quantity of exposures, environmental encounters could promote and attenuate the ongoing disease process during different stages of development (87, 88). Thus, environmental factors may act as triggers, accelerators and precipitators of the process leading to the clinical onset of diabetes.

The role of infections
Infections caused by at least 13 different viruses have been associated with type 1 diabetes in humans and different animal models (89-92). Viral infections may trigger the autoimmunity, or modify, accelerate and/or precipitate, the ongoing disease process (88, 93).
Mechanisms of action. There are three possible ways in which a virus could be involved in producing diabetes. Firstly, viruses can directly infect and rapidly destroy insulin-producing pancreatic beta cells causing acute diabetes without inducing autoimmunity. Genetically susceptible mice develop diabetes within 3 days after infecting them with high titre of D-variant encephalomyocarditis (EMC-D) virus (91). In humans this possibility is illustrated by a case report where coxsackie B virus was isolated from the pancreas of a child who died at the onset of type 1 diabetes (94), and by lesions found in the pancreas of children with fatal viral infections (95). Secondly, viruses may trigger or contribute to the autoimmune destruction of the beta cells by different mechanisms (91). In mice infected with a low dose of EMC-D virus the direct infection of the beta cells and macrophages is limited, and most of the beta cells are destroyed through apoptosis, which may be induced by interleukin (IL)-1β, tumour necrosis factor (TNF)-α and nitric oxide (NO) secreted by infection-activated macrophages. In diabetes resistant bio breeding (BB) rats Killham rat virus causes autoimmune diabetes without infection of beta cells by disrupting immune balance of Th1-like and Th2-like T cells, which leads to selective activation of beta-cell-cytotoxic T cells. A third possibility is that viruses, as all inflammatory events, can precipitate the clinical onset of the disease by increasing insulin need due to insulin resistance during infection (96, 97). This pathogenic mechanism is supported by population-based studies reporting significantly higher total amount of infectious diseases during the last year before the onset of type 1 diabetes in cases compared to controls (98-100), and case reports of simultaneous manifestation of type 1 diabetes after a flu-like infection in two members of the same household with varying residual beta cell capacity (101).

Epidemiological associations in humans. The most convincing evidence of an association between a viral infection and increased risk of type 1 diabetes in humans has been the finding of greatly increased risk of diabetes in individuals followed-up because of the congenital rubella embryopathy syndrome (102). Diabetes development among these patients is associated with HLA risk genes and presence of islet cell antibodies (103). In most Western countries vaccination programmes have now eradicated congenital rubella, however, by analogy some other viral infections could be similarly involved in beta-cell destruction and diabetes if experienced during the foetal life.

Enteroviral infections during the foetal life. Several small population-based case-control studies from Sweden and Finland found an association between maternal enteroviral infection during the pregnancy and increased risk of childhood type 1 diabetes in the offspring (104-107). However, an association between maternal enterovirus infection during the first trimester of pregnancy
and increased risk for type 1 diabetes in the child was not confirmed in a large Finnish material (108). In the prospective Finnish DIPP study enteroviral infections were frequently diagnosed during the pregnancy, but their incidence did not differ between cases and controls (15% in both groups) (109). This is at discrepancy with previous results, which could be due to different endpoints (diabetes vs. autoimmunity), different matching criteria (HLA-matched vs. non-matched), different epidemiological circumstances or chance due to small number of cases. Infections early in the child's life noted in the hospital records were found to be associated with an increased risk of diabetes, OR 1.61 (95% CI 1.11, 2.33), but no association was found with the common childhood infectious diseases (110).

**Enteroviral infections in newly diagnosed patients.** Several case-control studies showed that enterovirus antibodies (111-115) or enterovirus genome (116-120) are more often present in the serum of newly diagnosed type 1 diabetes patients compared to healthy control subjects.

**Prospective follow-up studies.** Only prospective follow-up studies may confirm the causal role of enterovirus and other infections in the initiation of the beta cell destruction and/or modification of the disease process leading to clinical diabetes. The first prospective studies were carried out in Finland as part of nationwide Childhood Diabetes study (DiMe), which follows siblings of patients with type 1 diabetes, and the Finnish type 1 Diabetes Prediction and Prevention (DIPP) trial, which follows from birth genetically susceptible children from the general population. The results from DiMe study suggested an association between enterovirus infections and the risk of type 1 diabetes (106, 121, 122). Also in the DIPP trial children who developed islet autoantibodies more often had an enteroviral infection during the 6-month period prior to the autoantibody appearance compared to those remaining autoantibody negative: 51% (21/41) and 28% (55/196), respectively (p=0.003) (109). Such specific clustering of enteroviral infections to the time of the first appearance of beta-cell autoantibodies among children matched for HLA-DQB1 risk genes and time of birth strongly supports a causal association. However, the numbers of the children developing autoimmunity and diabetes are still rather small, and the follow-up time relatively short. Two other prospective studies, German BABYDIAB (123) and American Diabetes autoimmunity study in the young (DAISY) (124) could not confirm the association between enterovirus infections and induction of islet cell autoimmunity. The discrepancies may depend from the differences of the length of sample intervals and the panel of enteroviruses used in screening tests, or due to differences between the study populations. A prospective study from Australia indicated a possible role for rotaviruses, but the role of enteroviruses was not analysed in detail in that study (125).
Cytomegalovirus. In a case report congenital cytomegalovirus (CMV) infection was also associated with subsequent development of type 1 diabetes (126). However, no association was found between serologically verified CMV infection during the pregnancy or during the last year before the diagnosis and subsequent development of type 1 diabetes in siblings of diabetic children in a case-control study (127).

Protective effect of infections. There is some evidence from animal experiments and indirect epidemiological associations in humans, suggesting that lack of exposure to infections early in life might increase the risk of getting diabetes. In genetically predisposed animals the frequency of diabetes increases if they are raised in pathogen-free environments (128, 129). Certain viral infections (130-132) or immune stimulation with bacterial antigens early in life significantly reduce diabetes incidence in these animals (133-136). Globally the highest and increasing incidence of insulin-dependent diabetes is observed in the affluent countries (3). A strong inverse correlation between total and childhood population density and incidence of type 1 diabetes was demonstrated in Finland (17). In Northern Ireland the incidence of insulin-dependent diabetes is lowest in areas with highest population density and most household crowding (25). A few case control studies from different countries have observed that diabetes risk is increased in children from higher socio-economic class families (137-139) and decreased in those living in overcrowded households (139) although the evidence is conflicting (100, 140, 141). These observations led to formulation of the ‘hygiene’ hypothesis, where exposure to infections early in life reduces the risk of diabetes (142-144). Pre-school day-care attendance, a proxy measure for total infectious disease exposure in early childhood, was also found to be inversely associated with diabetes (110, 145). A study based on prospective records in health care booklets showed that exposure to common childhood infections during the first year of life reduces diabetes risk (146). However, in a larger study based on maternal recall from structured questionnaires the protective effect was statistically non-significant (98).

Paradoxically, enteroviral infections were more frequent among healthy schoolchildren in Lithuania as compared to Finland, despite much lower incidence in the former (147). One explanation could be that when the frequency of enteroviral infections in the population decreases, an increasing proportion of infants lack protective maternal antibodies at the time they are exposed to enteroviruses, which could favour the initiation of autoimmunity to the beta cells (148). Indeed, due to differences in polio vaccination schedules, 9 month-old Finnish children, had a weaker cellular immunity against enteroviruses than Estonian children of the same age (n=21 in each group) (149). Thus, the lower incidence of IDDM in Estonia may partly be
explained by effective protection against diabetogenic strains of enteroviruses in Estonian children.

**Vaccinations**
In animal models Freund's complete adjuvant (CFA) and bacille Calmette-Guerin (BCG) vaccine modulate the development of type 1 diabetes – the proportion of NOD mice that develop diabetes is significantly reduced (133-136). However, no human data indicate such a protective effect. No significant difference was found in Sweden between birth cohorts with near-total BCG coverage during the newborn period before 1975 compared to cohorts born after the vaccination was completely stopped after 1976 (150). Neither was BCG vaccination associated with decrease in risk of type 1 diabetes in a case-control study (98). Only measles vaccination was associated with significant decrease in relative risk and thus showed a protective effect for type 1 diabetes, while vaccinations against smallpox, tetanus, whooping cough, rubella and mumps did not significantly affect the risk of developing type 1 diabetes in the same study (98). One study suggested a possible decrease of type 1 diabetes risk attributable to elimination of natural mumps by introducing mumps-measles-rubella vaccination in 1982 in Finland (151). However, the gradual increase in vaccination programmes does not permit any particular one to be pinpointed for the increase in type 1 diabetes. A recent study concluded that it was very unlikely that Haemophilus influenzae type b vaccination or its timing would influence diabetes risk in Finland (152). Similarly, the largest to date multi-center case-control study found no evidence that any common childhood vaccination modified the risk of diabetes (110). A recent analysis of two nationally representative British birth cohorts found that wild pertussis infection may be a risk factor for type 1 diabetes, and immunization may confer protection (153).

**Social and perinatal factors**
Pre-eclamptic toxaemia, Caesarean section (139) and maternal-child blood group incompatibility were found to be associated with increased risk of type 1 diabetes in case-control studies (154-156). It is possible that other not yet identified stressful events during the prenatal and perinatal period of life may also be risk factors for type 1 diabetes.

Several social characteristics of the family and material deprivation measures are associated with changes of type 1 diabetes risk in case-control studies e.g. older maternal age, mother having less than university education and father being a manual worker (99, 139, 140, 155-157). Most probably the mentioned social characteristics of the family effect the risk of type 1 diabetes by their association with other factors in the environment of the child, such as breast
feeding habits or exposure to infections, which are directly associated with pathogenesis of type 1 diabetes.

**Dietary factors in type 1 diabetes**

Dietary intake of certain nutrients and possible toxic food components is of interest in the search for triggers or promoters of the autoimmune β-cell destruction, which may lead to type 1 diabetes mellitus in childhood (87, 158, 159).

**Breastfeeding and cows milk.** The most extensively studied dietary exposures in the pathogenesis of type 1 diabetes are duration of breastfeeding and introduction of cow’s milk proteins. Animal experiments (160, 161) indicated that cow’s milk proteins might trigger type 1 diabetes, as the incidence of diabetes in these animal models significantly decreases if they are fed diets without intact cow’s milk proteins. In 1984 Borch-Johnsen and co-workers demonstrated that short duration of breastfeeding is a risk factor for type 1 diabetes in humans (162). Meta-analysis of population-based case-control studies found, that children with type 1 diabetes are more likely to have been breast-fed for < 3 months (overall odds ratio 1.43) and to have been exposed to cow’s milk before 4 months (overall odds ratio 1.63) than non-diabetic children (163). A few studies did not find an association between the duration of breastfeeding and the risk of type 1 diabetes or found a positive one (164-167), while the risk was considerably higher in genetically susceptible individuals (168, 169). In the Finnish DiMe study early introduction of dairy products was associated with increased diabetes risk independently of breastfeeding duration (170). Some case-control studies showed that high dietary intake of cow’s milk beyond the infancy period is an important risk factor for type 1 diabetes (100, 171).

More recently several prospective birth cohort studies have evaluated the association between the duration of breast-feeding and age at introduction of cow’s milk proteins with the development of beta-cell autoantibodies in infancy. In the Finnish DIPP study short-term exclusive breastfeeding for at least 4 months protected, and early introduction of cow’s milk based infant formula predisposed high-risk children form the general population to progressive beta-cell autoimmunity early in life (172). However, three other prospective birth cohort studies on first-degree relatives of patients with type 1 diabetes could not confirm such an association (173-175).

Most of the case-controls studies may have shortcomings because of their retrospective design (176), while the major disadvantage of prospective birth cohort studies is the small number of cases leading to low statistical power (only large increases in risk can be detected) (177). A double-blind randomised
dietary intervention trial will probably be needed to solve the controversies around the role of cow’s milk for the development of type 1 diabetes.

**Interpretation of the association.** The first interpretation of the observed associations was that breast milk protects the newborn against possible triggers, such as infectious agents (162), as it is well known that acute respiratory infections, diarrhoea and otitis media are lower in fully breast-fed compared to formula-fed infants (178-180). Alternatively, early introduction of cow’s milk proteins could be responsible. Studies in animal models (181) and newly diagnosed type 1 diabetes patients showed enhanced humoral (182-188) immune responses to different cow’s milk proteins compared to matched controls or non-diabetic siblings.

**The pathogenic mechanisms behind.** The pathogenic mechanisms explaining these associations are not clear, but molecular mimicry between cow’s milk proteins (bovine serum albumin, beta-lactoglobulin, beta-casein) and islet cell proteins (ICA69, retinal-binding protein, GLUT-2 glucose transporter) has been proposed. Recently a new hypothesis has been put forward, linking exposure to bovine insulin (BI) present in cow’s milk based formulas to the immunization against human insulin, the only known beta-cell-specific autoantigen in type 1 diabetes (189). The mechanism behind the association between short duration of breast-feeding and type 1 diabetes is still unsolved. If cow’s milk proteins are involved in the pathogenic process leading to type 1 diabetes, the pathogenic mechanisms may be related to the regulation of oral tolerance, thus gastrointestinal infections or other environmental factors that modify the normal gut bacterial flora may interact with the disease process (190). Indeed, coincident exposure to cow’s milk and enteroviral infection was associated with enhanced immunity to dietary insulin in infants with high HLA risk genotype (191).

**Other nutritional factors.** A population-based case-control study showed a dose-response relationship between the increased risk of developing childhood diabetes and the higher frequency of intake of foods rich in protein, carbohydrates and nitrosamines (192). Moreover, the nutrition-associated risk profiles differed between the age groups (193). In animal experiments (194, 195) as well as in human case-control (34, 196) and cohort (35) studies vitamin D supplementation was associated with reduced risk of type 1 diabetes. Nitrosamine compounds are toxic to beta cells in animal models of diabetes. The possible association in humans was first reported in the 1980s, by finding an association between consumption of smoked mutton, containing N-nitroso-compounds, and the incidence of childhood diabetes (197). Since then a few case-control studies (192, 198), and an ecological
association between the nitrate content in drinking water and the incidence of type 1 diabetes (199, 200) have supported this observation.

**Growth and development**

A large number of descriptive studies throughout Europe and worldwide have shown that type 1 diabetes incidence peaks in early puberty in both boys and girls (3, 5). This coincides with the timing of peak height velocity, when insulin resistance physiologically increases (201, 202), and the clinical onset of the disease may be accelerated by putting an extra workload for already decreased beta-cell mass. However, growth is associated with insulin demand even earlier in childhood. Already in the beginning of 1990’s a Swedish case-control study found that diabetic children, especially boys, were consistently taller and grew faster than the referent children several years prior to the clinical diagnosis of the disease, and rapid linear growth increased the risk for type 1 diabetes in childhood (203). Another Swedish study found that early weight gain was a risk factor for the development of type 1 diabetes (204). More recently higher body mass index or relative weight in addition to increased linear growth throughout childhood, but especially during the first three years of life, were shown to be risk factors for the development of type 1 diabetes (205-208). Differences in breastfeeding might be responsible for different growth rates and weight gain patterns early in infancy, as exclusively breast fed infants have a lower intake of energy resulting in a lower weight gain from three to nine months of age, compared to exclusively bottle fed from birth infants (209). Thus, lower weight gain in breastfed compared to non-breastfed children could partly explain the protective effect of breastfeeding against type 1 diabetes observed in several studies. However, one study found that even among exclusively breast fed children (>2 months), future diabetic cases gained significantly more weight from birth up to 18 and 30 months of age compared to referent children (204). A Finnish case-control study also concluded that early exposure to cow’s milk formula and rapid growth in infancy were independent risk factors of childhood type 1 diabetes (205). Although genetic predisposition towards rapid growth and susceptibility to hyperinsulinaemia cannot be excluded as underlying causes (71, 210), differences of energy intake probably play a role in promoting the more rapid growth of the pre-diabetic children.
AIMS

1. Variation of diabetes incidence in countries with different incidence levels and different socio-economic conditions may help understand the causes of diabetes. We compared the age-dependent incidence change, and possible variation of the incidence difference between Sweden and Lithuania according to age, including age span beyond 14 years at diagnosis. Time trend of childhood type 1 diabetes over an 18-year period (1983-2000) was also compared in the two countries.

2. During the last decades the incidence of childhood diabetes has increased in Sweden and in many other countries. However, it is not known whether diabetes is becoming more common in general, or if more patients are diagnosed at an earlier age. In order to shed light on these questions we studied incidence change during a 16-year period (1983-1998) in the age group 0-34 years in Sweden.

3. Type 1 diabetes has been associated with factors related to social class and wealth. During the past decade Lithuania has experienced a transition period, leading to dramatic socio-economic changes. Therefore, we studied type 1 diabetes incidence according to the urban-rural setting and the change over time according to age and sex in the age group 0-39 years during a ten-year period (1991-2000).

4. The role of infections in the aetiology of type 1 diabetes is complex – certain enteroviral infections may trigger autoimmunity to the beta cells, but lack of exposure to early non-specific infections may also increase the disease risk. Therefore, we studied whether the number of infections experienced during several periods from birth to diabetes diagnosis influenced the risk of diabetes.

5. Rapid growth, larger body size and more frequent dietary intake of certain nutrients have been associated with increased risk of childhood-onset type 1 diabetes. However, body size and energy intake are positively associated. Therefore, we studied the association between type 1 diabetes and previous intake of energy, accounting for the differences in body size. High intake of certain nutrients and foods, accounting for the total energy intake was also investigated.
MATERIALS AND METHODS

Description of Diabetes Registers (I - III)

Swedish Childhood Diabetes Study (age 0-14 years)

In the Swedish health care system all children aged 0-14 years with suspected diabetes are referred to paediatric departments. Since 1 July 1977 all 43 paediatric clinics in Sweden report newly diagnosed insulin-treated diabetes cases on a special form, including information about personal identification number, sex, county of residence, date of diagnosis (first insulin injection), date of reporting, reporting hospital and physician. Every 6 to 12 months the central register in Umeå requests the local contact person to verify and complete details about the recorded cases through the hospital medical records. The same method of data collection and verification was used since the start of the register. The register is approved for epidemiological analyses by the Swedish data inspection board and the ethics committee of the Karolinska Institute. Comparisons with external sources (13, 150) showed that the register included 96-99% of children 0-14 years old at diagnosis.

Diabetes Incidence Study in Sweden, DISS (age 15-34 years)

Since 1 January 1983 all departments of internal medicine (n=96), paediatrics (n=43), endocrinology (n=3) and more than 700 primary health care units in Sweden report new cases of diabetes mellitus aged 15-34 years at diagnosis. Information about personal identification number, name, address, sex, date of diagnosis, clinical classification of diabetes type (type 1, type 2, secondary, type unknown or not yet classified), date of reporting, reporting unit and physician, as well as some clinical characteristics are reported on a special form. Diabetes was diagnosed and classified according to clinical criteria as recommended by the WHO (7, 8). Reporting physicians classified the cases into diabetes types based on the clinical impression at the time of diagnosis. Insulin dependency, synonymous with type 1 diabetes, is judged to be present in cases with severe hyperglycaemia, ketosis, low or normal body weight and an immediate need of insulin therapy. Once a year the units who had reported at least one case during the last year receive a list of reported cases, and every unit receives a list of cases reported since the start of the register in 1983. Comparison with a computer-based patient administrative register in the two southernmost counties estimated that 86% of type 1 diabetes cases diagnosed 1983–1987 were included into DISS (211). A similar study in the county of Västerbotten estimated that 91% of cases diagnosed 1986–1997 were included (97% during 1986–1991; 86% during 1992–1997). The first study covered 9.2% and the second 2.9% of the Swedish population aged 15-34 years.
**Organisation of diabetes care in Lithuania**

*Children (0-15 years)* All children with suspected diabetes are initially treated in hospitals. The department of Childhood Endocrinology at the Kaunas University of Medicine is the only specialized department of childhood endocrinology in the entire country, where the majority of newly diagnosed cases are referred for initial treatment and periodical check-ups. For the provision of outpatient diabetes care for children with diabetes Lithuania is divided into five regions situated around the five largest cities, where paediatric endocrinologists work in the consulting polyclinics (Figure 2). Every three months children with diabetes living in the respective region – both in the city and in the small towns and rural areas of the region – visit their regional paediatric endocrinologist for the prescription of insulin.

*Adults (above 15 years)* Lithuania is divided into 44 administrative regions, with the largest town of the region being the administrative centre (Figure 2). Every administrative centre has a hospital and/or a consulting-polyclinic where an endocrinologist (in 3 regions a specialist of internal diseases) provides the care for patients with diabetes living in that region (both in the small towns and rural areas), and prescribes insulin, which is free of charge for diabetic patients. Since Lithuania is a small country, the distance to the administrative centre of the region is usually less than 30-40 kilometres. In the cities adult patients with diabetes are followed-up by endocrinologists from district polyclinics or specialised diabetes centres.

*Figure 2.* Division of Lithuania for the provision of outpatient diabetes care for children (5 larger divisions) and adults (44 smaller divisions).
Prospective registration of new childhood diabetes cases 14 years or younger at the time of diagnosis started in 1983, when a register centre was established at the Institute of Endocrinology, Kaunas Medical Academy. Newly diagnosed diabetic children were referred to the register centre for consultation and registration. In addition, regional paediatric endocrinologists prepared quarterly reports that were used as a complement of the primary data source. Yearly reports from the Ministry of Health of Lithuania were used as the secondary data source. The sources were not fully independent, as the same paediatric endocrinologists prepared both reports, however, no other sources were available. Department of Childhood Endocrinology was established at the Kaunas University of Medicine in August 1989. Since then the majority of newly diagnosed cases of childhood diabetes from the entire country are referred to this department for initial treatment, and constitute the primary data source of the register. Yearly reports of the regional paediatric endocrinologists are considered as the secondary data source. Due to the strict organisation of care for children with diabetes in Lithuania it is unlikely that cases are missing from the secondary data source, thus the ascertainment is estimated at 100% (10).

Lithuanian Register of type 1 diabetes in adults (age 15-39 years)

Since 1 January 1991 patients diagnosed with type 1 (insulin dependent) diabetes mellitus between 15 and 39 years of age and permanently residing in Lithuania are prospectively registered. All physicians responsible for outpatient care of people with diabetes from territorial health care units throughout the country (n=106) report new cases with type 1 diabetes to the register centre on a special form, deemed mandatory by the Lithuanian Ministry of Health “Report about persons with newly diagnosed diabetes mellitus”. Information including personal identification code, name, date of birth, gender, address, date of clinical diagnosis, date of first insulin injection, date of reporting, reporting unit and physician, and some clinical characteristics (ketonuria and/or acidosis, blood glucose value at the time of diagnosis) are registered for every patient. Diabetes was diagnosed and classified according to clinical criteria as recommended by the WHO (7). Only those cases where insulin therapy was initiated at, or within two weeks of diagnosis, and lasted for at least several months, were regarded as having type 1 diabetes and included into the register. In the beginning of the year every reporting physician is asked to complete three lists and return them to the register centre: 1) List of follow-up of all insulin-treated diabetic patients; 2) List of newly insulin-treated diabetic patients (diagnosed during the previous year); 3) List of insulin treated diabetic patients removed from the follow-up. New cases reported throughout the previous year are then checked against the
lists in order to verify that they are still treated with insulin, and find the cases that were missed. These lists are considered the secondary source of ascertainment together with records of the causes of death from the Lithuanian department of Statistics (including death certificates), and membership lists from the Diabetes Societies. The overall completeness of case ascertainment was estimated at 91% during 1991-1997 (212).

**Table 1. Register material used in different studies**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Country</th>
<th>Time period</th>
<th>Cases (n)</th>
<th>Average population</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>0-14</td>
<td>Sweden &amp; Lithuania</td>
<td>1983-2000</td>
<td>4171</td>
<td>3860</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>543</td>
<td>557</td>
<td></td>
</tr>
<tr>
<td>0-34</td>
<td>Sweden</td>
<td>1983-1998</td>
<td>6716</td>
<td>5035</td>
<td>II</td>
</tr>
<tr>
<td>0-14</td>
<td>Birth cohort</td>
<td>1978-1982</td>
<td>1002</td>
<td>841</td>
<td>III</td>
</tr>
<tr>
<td>0-39</td>
<td>Lithuania</td>
<td>1991-2000</td>
<td>1072</td>
<td>748</td>
<td></td>
</tr>
<tr>
<td>0-34</td>
<td>Sweden &amp; Lithuania</td>
<td>1991-1998</td>
<td>3460</td>
<td>2680</td>
<td>New</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>918</td>
<td>690</td>
<td></td>
</tr>
</tbody>
</table>

**Statistical methods (papers I-III)**

Population data in the relevant age groups of males and females were obtained from Statistics Sweden and the Lithuanian Department of Statistics. Age and sex specific incidence rates per 100,000 and year (papers I-III) and cumulative incidence per 100,000 (paper II) were calculated. Ninety-five percent confidence intervals for the incidence rates were estimated assuming Poisson distribution of the cases. Direct age-standardisation of the incidence rates was performed assuming a standard population with equally sized five-year age groups of both sexes. Poisson regression analysis was performed using Egret for Windows (CYTEL, Cambridge, MA, USA).

**Paper I.** Incidence change over time in Sweden and Lithuania was studied as percent average change per year from 1983 and 2000 according to sex and age group. Average incidence rates were also compared during two periods (1992-2000 relative to 1983-1991) by calculating incidence rate ratios. To check whether the incidence change during the two periods differed between the age groups, we studied the interaction between age group and period.
Table 2. Lexis diagram: relation between calendar time period (year of diagnosis), age at diagnosis and birth cohort (year of birth) in the Swedish register data, used in the analysis of the period and birth cohort effects (paper II).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Year of diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>1978</td>
</tr>
<tr>
<td>19</td>
<td>1979</td>
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<tr>
<td>20</td>
<td>1980</td>
</tr>
<tr>
<td>21</td>
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<td>1982</td>
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<td>37</td>
<td>1997</td>
</tr>
<tr>
<td>38</td>
<td>1998</td>
</tr>
</tbody>
</table>

Note: The diagram is a visual representation of the Lexis diagram, showing the relationship between age at diagnosis and birth cohort.
**Paper II.** Table 2 shows the availability of data according to the age at diagnosis, calendar time (year of diagnosis) and birth cohort (year of birth) and their inter-relationship (Lexis diagram).

**Time period effect.** For the Poisson regression analyses age was categorised into twelve 3-year age groups and calendar time into four 4-year periods (1983-1986, 1987-1990, 1991-1994, 1995-1998). Boys, 0-2 years age group and 1983-1986 years calendar period were used as reference categories.

**Birth cohort effect.** Age-specific incidence rates per 100,000 live births for five successive 10-year birth cohorts – people born in the 1950s, 1960s, 1970s, 1980s and 1990s were calculated. Diabetic people were born between 1948 and 1998: 0-14-year old between 1963 and 1998, 15-34-year old between 1948 and 1983. As can be seen in Table 2, different birth cohorts covered a varying span of age at diagnosis where full data was available: people born 1950-1959 had full data for age span 25-34 years, 1960-1969 – 10-34 years, 1970-1979 – 0-26 years, 1980-1989 – 0-16 years and 1990-97 – 0-6 years, respectively. Every age category in the birth cohorts was represented by mean incidence per 100,000 live births during a 2 to 10-year time interval (age categories represented by only one year-of-birth were excluded). For example, for the 14-year old people mean incidence per 100,000 live births during 1964-1969, 1970-1979 and 1980-1983 was calculated.

**Paper III.** For the comparison of incidence rates by the urban-rural setting we used the classification of the Lithuanian Department of Statistics, which is based on Lithuanian legislation, defining towns as “compactly built-up territories having at least 3000 inhabitants with more than 2/3 of them employed in non-agricultural occupations”. According to this definition there were 114 towns in 1991 and 106 in 2000 in Lithuania (the number of towns changed due to administrative reform). Age was categorised into 5-year and 10-year age groups, calendar time into two 5-year periods (1991-1995, 1996-2000) and the degree of urbanisation into three categories (cities, including 5 largest cities with >100,000 inhabitants; towns, including all other towns except 5 cities; and rural areas). Incidence in females, youngest age group, 1991-1995 years calendar period, and rural areas were used as reference.
Description of Case-Control studies (IV - V)

The Lithuanian study (IV)

The study material was collected as an additional data set within a multi-centre European study EURODIAB sub-study 2 “Environmental Determinants of Insulin-dependent Diabetes in Childhood”. The Ethics Committee of Kaunas University of Medicine approved the Lithuanian part of the study. Informed consent was obtained from the parents of study participants for checking medical documentation. Diabetic patients were selected from the Lithuanian Childhood Diabetes Register. As there was no computerised population register in Lithuania at that time, a two-stage sample selection procedure was used to form a population-based control group. The overview of the selection procedure of the study participants, the response rates and reasons for non-response are shown in Figure 3.

- Cases diagnosed in Lithuania 1989-1994 n=368
- Cases in 5 cities 1989-1994 n=164
  - Born 1980-1994 n=130
  - Born 1975-1979 n=34
  - Emigrated n=4
  - Died n=2
- Non-responders n=7 (6%)
  - Active refusal n=2
  - Passive refusal n=5
- Responders n=117 (94%)
- Selected control children n=372
  - Randomly selected district paediatricians in 5 cities
    - 53 of 475
    - 34,482
  - Refusal
    - Active n=17 (4.6%)
    - Passive n=19 (5.1%)
  - Other reasons
    - No trace n=54 (14.5%)
    - Left study area n=12 (3.2%)

Figure 3. Overview of selection and participation of cases and controls in the Lithuanian case-control study (Paper IV)
An overview of the data material is presented in Table 3. A questionnaire inquiring about a variety of environmental exposures was prepared for the EURODIAB sub-study 2 according to a common protocol, adding questions about the socio-economic situation of the family at the time of child’s birth, and the duration of parental education. A total of 117 (94%) cases and 270 (73%) controls returned the questionnaires. There were no systematic differences between the responding and non-responding diabetic and control children regarding their age group and sex. In the 0-4 years group responding control children were younger compared to patients (Mann-Whitney test, two-tailed p=0.048). Information about infectious diseases and feeding in infancy was collected from health care booklets of the responding study participants (available for over 90%). Number of infections experienced during the first half-year, the last year before the onset, and from birth until the onset of diabetes (or 1 January 1992 for the controls) were analysed with respect to diabetes risk. End of breast-feeding was estimated as the mid-point between the last record with breast-feeding and the first record without breast-feeding.

Table 3. Methodological overview of the case-control studies.

<table>
<thead>
<tr>
<th>Study setting</th>
<th>Subjects</th>
<th>Description of subjects</th>
<th>Data and data sources</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LITHUANIA</strong></td>
<td>Cases</td>
<td>Diagnosed 1989-1994</td>
<td>Infections from birth to diagnosis, breast-feeding (prospectively recorded in health care booklets and birth hospital records)</td>
</tr>
<tr>
<td>5 cities</td>
<td>117 of 124</td>
<td>Aged 0-14 years</td>
<td></td>
</tr>
<tr>
<td>(Paper IV)</td>
<td>Controls</td>
<td>Population-based</td>
<td>Mailed questionnaires (education of parents, socio-economic situation)</td>
</tr>
<tr>
<td></td>
<td>270 of 372</td>
<td>Two-stage selection</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>At group level matched for sex and age</td>
<td></td>
</tr>
<tr>
<td><strong>SWEDEN</strong></td>
<td>Cases</td>
<td>Incident cases</td>
<td>Dietary intake one year before the diagnosis (dietary history interview using food frequency questionnaire)</td>
</tr>
<tr>
<td>Stockholm</td>
<td>99 of 100</td>
<td>Aged 7-14 years</td>
<td></td>
</tr>
<tr>
<td>(Paper V)</td>
<td>Controls</td>
<td>Population-based</td>
<td>Routinely recorded growth data (Child Health Clinics and Schools)</td>
</tr>
<tr>
<td></td>
<td>180 of 200</td>
<td>From population register</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Individually matched for age, sex and geographical region</td>
<td></td>
</tr>
</tbody>
</table>
Statistical methods. Variables were categorised using the percentile values estimated from the distributions of their values in cases and controls taken together. Number of early infections, number of people in the household and duration of mother’s education were categorised using the value of the 50th percentile, duration of breast-feeding - using the 25th percentile for cut-off. Number of infections experienced from birth to diabetes onset was categorised separately in the 0-4 and 5-14 years groups, using the value of the 50th percentile of the age group, because the number of infections markedly differed between the groups. Adjustment for the duration of follow-up in years was made when analysing the association between the total number of infections and diabetes risk. Analyses were performed using the QUEST software (developed by L. Gustafsson, University of Umeå, Sweden) and Statistical Package for Social Sciences (SPSS 7.5.1). Logistic regression analysis was used to adjust for potential confounders by entering combinations of variables into the models.

The Swedish study (V)

The Ethics Committee at the Karolinska Institute and the Swedish Data Inspection Board approved the study. Ninety-nine of 100 incident cases of diabetes mellitus 7-14 years-of-age at diagnosis occurring in Stockholm area, and 180 of 200 control children matched for age, sex and geographical region identified through the official Swedish population register agreed to participate.

Dietary data. Specially trained dieticians performed the interviews with the child and one or both parents regarding the dietary intake one year before the diabetes diagnosis (one year before the interview for the control children). The dietary history method started by mapping the general food habits, meal frequencies, meals at home, at school and elsewhere, and thereafter advanced into meals and general dietary intake. The school menu for the interview period was used as a reference. A system of cross checking to the meal pattern and number of breakfasts, lunches and dinners per week was used throughout the interview. Consumed amounts were estimated using household measures and pictures of portion sizes. Frequencies and amounts of consumed foods were recorded in a food frequency questionnaire including 206 different foods and dishes. The same dietician interviewed the case and matched control children. Average individual intake of certain foods or food groups was calculated and expressed as grams per day. Dietary data were transformed into energy and nutrient intake per day using the Swedish Food Data Bank, computerised by the Swedish National Food Administration.
Growth data. After permission from the parents, prospectively recorded growth data were retrieved from the records at Child Health Clinics and School Health services. Growth data before the diagnosis (or interview for the controls) were available for 77 (78%) cases and 148 (82%) controls with 5.7 and 5.1 measurements per individual on average, respectively (p=0.54). The proportion of cases and controls without growth data did not differ (25 and 21 percent respectively, p=0.37). Growth data were transformed into age- and sex-specific standard deviation scores (SDS) according to the 1978 CDC/WHO reference (213), used by the EpiNut program of the EpiInfo software (EPI Info 6.1, Atlanta, Georgia, USA). To represent the relative body size of the individual we calculated an average SDS of measurements taken during the four-year period preceding the diagnosis or interview, excluding the measurements during the last three months prior the diagnosis. Seventy-five cases and 144 controls had growth data available, with 2.3 and 2.5 measurements on average, respectively (p=0.22). Dietary analysis was possible for 98 matched sets of case and referent children: 78 with two, and 20 with one referent child per case. Analysis including both dietary and growth information was possible for 67 matched sets (67%): 40 with two and 27 with one referent child per case.

Statistical methods. Mean intake of energy and different nutrients, and mean standard deviation scores of cases and controls were compared using the one-way analysis of variance. Conditional logistic regression analysis of matched case-referent sets was performed using EGRET software (Epidemiological Graphics Estimation and Testing Packages, Statistical and Epidemiological Research Corporation, Seattle, Washington, USA). For the logistic regression analyses of different nutrients and energy (98 matched sets), the intake of energy and nutrients were dichotomised using the 75th percentile. For the analyses of diet and growth (67 matched sets), the intake of energy and average SDS were grouped into three levels of exposure using the values of the 33rd and 66th percentiles of the distribution in cases and controls for cutoff: low (<33rd), medium (33-66th) and high (>66th).
RESULTS

Descriptive epidemiology

*Incidence difference in Sweden and Lithuania (I, unpublished)*

The average diabetes incidence in the 0-14-year age group during the period 1983-2000 was almost four times as high in Sweden as in Lithuania, 28.9 and 7.5 per 100,000 per year, respectively (IRR (95%-CI)=3.8 (3.6-4.1)). Considering the age-dependent incidence change, we compared the age- and sex-standardized type 1 diabetes incidence between Sweden and Lithuania in the 0-34-year age group during the period 1991-1998/2000 (Figure 4). Incidence difference varied significantly with age (p<0.001), being largest in the youngest age group and attenuating with increasing age. Compared to Lithuania, the incidence was 4.9 times (4.0-6.0) higher in 0-4-year children in Sweden, while there was no difference in 30-34-year old people between the two countries (IRR=0.98 (0.84-1.13)). Accordingly, the difference of age and sex-standardized incidence between Sweden and Lithuania was 2.6 times (2.3-2.8) larger in the 0-14-year compared to the 15-34 year age group (IRR_{0-14}=3.7 (3.4-4.1); IRR_{15-34}=1.4 (1.3-1.5)). The association was similar in males and females.

*Figure 4.* Incidence rate ratio according age in the 0-34-year age group in Sweden (1991-1998) relative to Lithuania (1991-2000)
**Age-dependent incidence change (I - III)**

In both countries incidence increased with age and peaked in association with puberty, about two years earlier for girls compared to boys, and decreased thereafter (Figure 5 and Figure 6). During the post-pubertal years the incidence was higher in males compared to females in both countries. Incidence did not differ between the 0-14-year old boys and girls (Sweden, $p=0.25$; Lithuania, $p=0.29$), but it was higher in males than in females in both countries when including a broader age span: 21.4 and 17.1 (IRR=1.25, $p<0.001$) in the 0-34-year age group in Sweden, and 9.5 and 6.9 (IRR=1.39, $p<0.001$) in the 0-39-year age group in Lithuania, respectively.

**Figure 5.** Incidence of type 1 diabetes per 100,000 per year in 0-34-year old Swedish males and females 1983-1998

**Figure 6.** Incidence of type 1 diabetes per 100,000 per year in 0-39 years old Lithuanian males and females 1991-2000, presented as 3-year moving averages
Incidence change over time in the 0-14-year group in Sweden and Lithuania (I)

Between 1983 and 2000, the average increase per year was 2.2% in Sweden (95%-CI 1.7-2.6) and 2.3% in Lithuania (95%-CI 1.1-3.5), but the latter trend depended on an increase during the last few years of the period, and only for girls (Figure 7). In Sweden, incidence increased significantly in all age groups, more so in the younger groups (3.0%, 2.2% and 1.7% per year in 0-4, 5-9 and 10-14-year age groups, respectively), while in Lithuania a significant increase was found only in the 10-14-year age group (3.0%). In Sweden a trend towards a younger age at diagnosis was indicated for both boys (p=0.06) and girls (p=0.01) when comparing 1983-1991 and 1992-2000, whereas in Lithuania the changes in age distribution over time were small, with an opposite tendency for boys (p=0.01).

Figure 7. Age-standardized type 1 diabetes incidence per 100,000 per year in 0-14-year old children in Sweden and Lithuania during 1983-2000 (3-year moving averages). Reference lines show average incidence during the entire period.
**Incidence change over time in the 0-34-year group in Sweden (II)**

Between 1983 and 1998 the incidence increased over time in boys and girls aged below 15 years in Sweden, but it tended to decrease at older ages, especially in men. Cumulative incidence at 35 years was rather stable during the four 4-year periods in males, while in females it varied more (Figure 8). In males the age-adjusted incidence in the 0-34-year age group did not differ between the 4-year periods (p=0.63). Compared to 1983–1986 the incidence was 2% (p=0.54) higher in 1995–1998. However, the pattern of change by the time period differed between the 3-year age groups (p<0.001), indicating a decrease of age at diagnosis over time. In females the incidence in the 0-34-year age group differed between the periods (p<0.001). Compared to 1983–1986, the incidence was 8% (p=0.03) lower in 1987-1990, and tended to increase by 7% (p=0.10) in 1995–1998. The pattern of change over time, however, did not differ significantly between the 3-year age groups (p=0.08). Thus, no statistically significant increase in incidence has occurred in the 0-34-year age group in Sweden over the 16-year study period.

![Figure 8. Cumulative incidence of type 1 diabetes per 100,000 in 0-34 years old Swedish males and females during four 4-year periods 1983-1998 (1983-86 (solid line); 1987-90 (- - -); 1991-94 (---); 1995-98 (--------)).](image-url)
**Incidence by birth cohort in Sweden (II)**

In both boys and girls below 14 years of age at diabetes diagnosis the incidence increased in successive birth cohorts (Figure 9). Compared to 0-6-year old children born during the 1970s, the incidence was higher for children born during the 1980s (IRR 1.11; 95% CI 1.01-1.22) and 1990s (IRR 1.47; 1.33-1.62). For 7-14-year old children, the incidence was higher for children born in the 1980s (IRR 1.19; 1.12-1.26) than in the 1970s, while it was lower for those born in the 1960s (IRR 0.89; 0.82-0.97) than in the 1970s. However, no clear birth cohort effect could be seen in the older age groups, 15-34 years at diagnosis (p=0.23), although there was a tendency of higher incidence in the birth cohorts of 1950s and 1960s compared to the 1970s.

Figure 9. Age-specific incidence of type 1 diabetes per 100,000 live births in 0-34-year old Swedish males and females born during the 1950s, 1960s, 1970s, 1980s and 1990s.
**Urban-rural differences of diabetes incidence in Lithuania (III)**

In Lithuania, the incidence of type 1 diabetes differed depending on the urban-rural setting. The age- and sex-standardized incidence in the group aged 0-39 years was lower in the rural areas than in small towns and cities (7.1, 9.0 and 8.8 per 100,000 per year, respectively). Compared to the rural areas, the incidence was 1.22 (p<0.001) times higher in the cities and 1.27 (p<0.001) times higher in small towns. The urban-rural gradient of incidence was most evident in the younger age groups (Figure 10).

![Figure 10. Urban-rural differences of type 1 diabetes incidence per 100,000 inhabitants per year in 0-39-year-old Lithuanian males and females 1991-2000](image)

**Change over time in the 0-39-year group in Lithuania (III)**

Compared with 1991-1995, the incidence of type 1 diabetes was higher during 1996-2000 for people aged 0-39 years at diabetes diagnosis. The age-standardized incidence per 100,000 inhabitants per year increased from 8.7 to 10.5 for the males (IRR=1.22, p=0.001), and from 6.2 to 7.8 for the females (IRR=1.25, p=0.002). For males, the increase over time occurred predominantly in the cities from 8.4 to 11.8 (IRR=1.40, p<0.001), and in the older age groups. In contrast, for females, the incidence increased more in small towns and rural areas, 5.8 to 7.7 (IRR=1.33, p=0.003), and in the younger age groups.
Analytical epidemiology (case-control studies)

Protective effect of early infections (IV)
Exposure to one or more non-specific infection during the first half year of life was associated with reduced diabetes risk. After adjustment for duration of breast-feeding, number of people in the household, duration of mother’s education and birth order of the index child odds ratios (95% confidence intervals) in the 0-14, 0-4 and 5-14-year age groups were 0.60 (0.37-0.98), 0.94 (0.40-2.20) and 0.47 (0.26-0.87), respectively. The number of infections recorded during the last pre-onset year or from birth to onset did not influence diabetes risk. There was, however, a tendency for increased risk in the 0-4-year age group if children were exposed to more infections.

The effect of diet and growth (V)
Average intake of energy and nutrients, as well as mean weight-for-age standard deviation score (SDS), was significantly higher in cases compared to controls. After adjustment for each other, higher previous weight-for-age and higher intake of energy were both associated with increased risk of type 1 diabetes (Figure 11). When accounting for high total energy intake, high intake of carbohydrates, odds ratio (95%-CI) 3.06 (1.24-7.56), particularly high intake of disaccharides (2.37 (1.14-4.93)) and sucrose (2.16 (1.15-4.05)), increased the risk of diabetes.

Figure 11. Intake of energy and mean weight-for-age standard deviation score during the last four years before the diagnosis, and the risk of type 1 diabetes in 7-14-year old Swedish children (67 matched sets).
The combined effect of total energy intake with intake of energy from fat was also investigated. Compared to Low energy-Low fat diet, diabetes risk was about twice as high for children on either Low energy-High fat, or High energy-High fat diets (Figure 12). However, there was a further increase in diabetes risk if high intake of energy was combined with low intake of energy from fat.

![Figure 12. Odds ratios for type 1 diabetes: combined effect of total energy intake with intake of energy from fat in 7-14-year old Swedish children (98 matched sets). Levels of exposure: Low (<75th percentile); High (>75th percentile).](image-url)
Summary of the results

1. The largest difference of type 1 diabetes incidence between Sweden and Lithuania was found in the younger age groups, and the difference decreased with age.

2. The age-dependent pattern of incidence change below 20 years at diagnosis was similar in both countries, as well as incidence differences between the males and females.

3. Within a low-incidence country Lithuania, type 1 diabetes incidence differed depending on the urban-rural setting. The urban-rural gradient of incidence, that seemed to follow the differences of the poverty distribution, was also most evident in the younger age groups.

4. In Sweden the largest relative increase over time in childhood age range is currently occurring in the youngest age groups. The findings in the age group below 35 years also indicated that diabetes is not becoming more common, but rather more people are being diagnosed at an earlier age. Analysis of 10-year birth cohorts showed a gradual increase in incidence over time – first in the older children, later also in the younger. The follow-up period was, however, too short to confirm the shift towards a younger age at diagnosis in the birth cohort material.

5. An increase in both younger (<15 yr) and older (>15 yr) age groups has occurred during the 1990s in Lithuania. When analysing the change over time in the childhood age range since the early 1980s, the incidence has increased significantly in the older children (10-14 years) only. Thus no “shift” toward a younger age at diagnosis was indicated in Lithuania.

6. Exposure to infections early in life may decrease childhood diabetes risk, particularly for children diagnosed after the age of 4 years.

7. Higher energy intake and higher previous relative body weight seem to be independently associated with increased diabetes risk. Of the different nutrients, high intake of carbohydrates, disaccharides and sucrose in particular, increase the risk. High intake of energy from non-fat sources seems to be unfavourable with respect of type 1 diabetes risk.
DISCUSSION

General discussion

It is currently agreed that the aetiology of type 1 diabetes is complex and multi-factorial. Genetic predisposition is required, but in itself not sufficient, for the disease to develop. Thus, environmental factors are believed to be involved as triggers, modifiers and promoters of the autoimmune process leading to clinical diabetes (87, 88).

The incidence of childhood type 1 diabetes varies considerably throughout the world (4) and in Europe (10), as well as in countries in the region of the Baltic Sea (14), including Lithuania and Sweden (paper I). The causes of such a marked geographical variation are not fully understood, but may result from different distributions of genetic (214, 215) or environmental determinants, or both, in different populations. However, in Lithuania (paper III), as well as in several other countries (17, 22-26), incidence differs depending on the urban-rural setting, or population density. Such variation of incidence within the same country supports the importance of environmental factors. The epidemiology of type 1 diabetes beyond 14 years-of-age is less extensively studied, although the data available suggest that incidence levels in different countries vary less in the 15-29-year, compared to the 0-14-year age group (see Figure 1 in the introduction). We have found that type 1 diabetes incidence between Sweden and Lithuania differed most in the younger age groups, and the difference attenuated with age (unpublished results). This observation supports the hypothesis of a “shift” towards a younger age at diagnosis in the high-incidence countries.

During the 1990s the incidence of childhood type 1 diabetes has increased in almost all regions in Europe, particularly rapidly in the former socialist countries of Central Eastern Europe (10), and the relative increase was more rapid in the younger age groups. Also in the current study (I) the relative increase over an 18-year period was larger in the younger age groups in Sweden, while in Lithuania a significant time trend was found in older children only. Very few studies have analysed time trend of diabetes incidence in the age groups beyond 14 years at diabetes diagnosis, but the results differ in different countries. An increase in both younger (<15 yr) and older (>15 yr) age groups has occurred in Lithuania during the 1990s (paper III). Similarly, a tendency of an increase was found in both children (0-14 y) and young adults (15-29 y) in Turin (Italy) 1984 through 1996 (52). In West Yorkshire, the incidence tended to increase in the 0-14, and was stable in the 15-29-year age group between 1991 and 1999 (53). However, in Belgium (28), as well as in Sweden (paper II), the change of incidence over time seemed to have opposite
directions for childhood-onset and adult-onset type 1 diabetes, supporting a shift towards an earlier age at diagnosis rather than an overall increase in incidence.

There is no simple explanation as to why temporal changes in the incidence of type 1 diabetes occur in some countries and are absent in the others. Rapid incidence changes over time support the role of environment in the aetiology of type 1 diabetes. A recent analysis of published data from different populations throughout the world found, that the magnitude of the increase in incidence was negatively associated with the incidence rate in the population, suggesting a more rapid increase in the low-incidence countries (216). However, the factors determining incidence change over time probably are more complex, as also suggested by our results.

The risk of childhood diabetes has been previously shown to be positively associated with estimates of wealth. This has been shown over time in Sweden (38). Also among different countries in Europe the levels of incidence and wealth are positively associated (31). Further, in some countries (25, 137) the incidence of childhood diabetes is lower in areas that are materially more deprived. Also in Lithuania (paper III) the observed urban-rural gradient of type 1 diabetes incidence during the 1990s paralleled the urban-rural differences of the poverty distribution: 26-28% of people in the rural areas, 15-14% in small towns, and 10-7% in the cities during 1997-1999 were reported to live below the relative poverty line, defined as consumer expenditure per household member below 50 percent of the countries average (217). Associations at the population level should be interpreted with caution, as they may be misleading. Nevertheless, differences in the distribution of wealth-related risk factors, and the way these factors change over time for the majority of the population, may partly explain both the different incidence rates, and differences of incidence change over time between the populations. It may be speculated that the relatively recent start of the small increase in the childhood diabetes incidence in Lithuania seen since the early 1980s may be explained by unfavourable socio-economic changes that affected a large part of the population during the 1990s (218).

It is not known which risk factors for childhood diabetes are associated with wealth or Westernisation of lifestyle. Development of all types of diabetes probably depends on an interplay of insulin deficiency and insulin resistance (219), thus, changes of insulin sensitivity during the pre-diabetic period may be important in determining the time of the clinical presentation in type 1 diabetes (219-221). Indeed the highest age-specific incidence for both boys and girls in most countries, including Sweden (paper II) and Lithuania (paper III), coincides with the timing of puberty and age at peak height velocity,
when insulin resistance physiologically increases (201, 202). This also explains why the age of peak incidence has changed little over the years in Sweden (paper II), although the age at diabetes diagnosis has decreased. If environmental risk factors promoting the disease process, or precipitating the diagnosis by increased insulin demand, have become more prevalent over the years, or the timing of the exposure to such factors has become earlier, this could have led to a clinical presentation of diabetes at an earlier age without increasing the total risk of the disease.

The accelerator hypothesis argues that the rising incidence of both type 1 and type 2 diabetes is related to increasing insulin resistance due to weight gain and physical inactivity (87, 222). Mean BMI at the age of seven years has increased significantly for both sexes in Stockholm schoolchildren born 1963 through 1983 (223). In a large population-based study a higher BMI gain between the age of 2 and 8 years was related to an increased gain in height during the same period, an earlier onset of puberty and less height gain in adolescence (224). One might speculate that a smaller proportion of children in the poorer rural areas in Lithuania are overweight, and they probably also are more physically active, which should lead to less insulin resistance.

Several studies in different populations, including both Sweden and Lithuania, have shown that more rapid linear growth and weight gain, as well as increased weight-for-height or body mass index throughout childhood, perhaps especially during the first three years of life, are associated with an increased risk of childhood diabetes (203-207, 225). Although genetic predisposition towards rapid growth or susceptibility to hyper-insulinaemia may be the underlying causes (71, 210), still, differences of energy intake probably also play a role in promoting the more rapid growth of the pre-diabetic children. In accordance with another study based on mailed food frequency questionnaires (192), we found, that higher energy intake and higher relative body weight seem to be independently associated with increased diabetes risk (paper V).

In childhood, over-nutrition leads to accelerated growth with respect to height and excess weight gain, both associated with a higher insulin demand and an increased workload for the beta cells. This may precipitate the clinical diagnosis by unmasking insulin deficiency, but also accelerate the process of autoimmune destruction, as functionally stressed beta-cells are immunologically up-regulated (226) and may be more sensitive to the toxic effects of cytokines (227). Thus, more rapid growth and excess weight gain due to improved nutrition, or even over-consumption during infancy and childhood, could be one of the wealth-associated risk factors responsible for the increasing incidence of childhood diabetes observed in many countries.
Our study may indicate that type 1 diabetes is not becoming more common, at least not in the high-incidence country Sweden by the age of 35 years, but more cases are diagnosed at an early age. However, this conclusion should be taken with caution, as it is difficult to estimate how much would the results of the current study be influenced if it were possible to include all cases with LADA in the 15-34 years group, or register the incidence of type 1 diabetes even after the age of 34 years. In Sweden childhood type 1 diabetes incidence has increased in successive birth cohorts. The most marked increases seem to have occurred for the 7-14 years old children born during the 1980s and for the 0-6 years old children born during the 1990s. However, no birth cohort extends over the whole age span yet, and the cohort born during the 1980s could only be followed until the age of 16 years. Thus, it is not clear whether the increased risk of type 1 diabetes in the young birth cohorts will be carried-on for life (supporting increased initiation of autoimmunity against the beta-cells), or whether there would only be a downward shift in the age at diagnosis (supporting acceleration of the pathogenic process), or both.

Another risk factor associated with socio-economic conditions is exposure to infections. The hygiene hypothesis suggests that early contact with microbial antigens may prevent autoimmune diabetes (142, 143). In genetically predisposed animals the frequency of diabetes increases if they are raised in pathogen-free environments (128, 129). Certain viral infections (130-132) or immune stimulation with bacterial antigens early in life significantly reduce diabetes incidence in these animals (133-135). Some case-control studies, including our own (paper IV), have found that exposure to non-specific infections during the first year of life is associated with a reduced risk of childhood diabetes (146). In a study based on a larger material from structured questionnaires the protective effect was statistically non-significant (98). Interestingly, although enteroviruses may be involved in the initiation of autoimmunity to the beta cells (109), it was shown that enteroviral infections were more frequent among healthy schoolchildren in Lithuania as compared to Finland despite a lower diabetes incidence in the former (147). One explanation could be that when the frequency of enteroviral infections in the population decreases, an increasing proportion of infants lack protective maternal antibodies at the time they are exposed to enteroviruses, which could favour the initiation of autoimmunity to the beta cells (148).

The higher incidence of type 1 diabetes in males than in females after the puberty, and different pattern of incidence change over time between the sexes (III) indicate either different life-style related risk factors, or their different distribution between males and females. In a national sample, investigated in 1997, the prevalence of obesity among 20-34 years old Lithuanians was 6% for both sexes, but the prevalence of overweight was
higher in males than in females, being 34% and 19%, respectively, while 8% of females, but no males were underweight (228). These differences in the prevalence of overweight are consistent with our findings of a greater increase of diabetes incidence between men compared to women in the corresponding age groups. Interestingly, during the 1980s mean BMI increased most in the 25-34 years age group in Sweden and more for women (229) than for men (230), which could, perhaps, partly explain why the decrease of type 1 diabetes incidence was more pronounced in the 15-34 years old males than in females (II).

Conclusion

Taken together, the findings presented in the current thesis suggest, that environmental factors associated with socio-economic conditions and wealth, perhaps particularly during the early childhood, may be important for the occurrence of type 1 diabetes. This possibility is also supported by the results of the case-control studies, as exposure to infections, nutrition habits and growth pattern are known to differ depending on the socio-economic conditions in childhood. Life style habits leading to less exposure to microbial antigens early in life, and to a high intake of energy and higher growth rate in childhood may contribute to the increase of childhood-onset type 1 diabetes.
Method problems in the register studies (I-III)

Ascertainment (capture-recapture method)

All four diabetes registers used in the studies presented in this thesis used two data sources, and the completeness of the case ascertainment was estimated at 90% or more, as generally recommended for all diabetes registers (30, 231-233). In both countries the level of ascertainment was around 10% higher in the age group 0-14 years than >14 years. The ascertainment in the Swedish childhood and adult type 1 diabetes registers was estimated at 96-99% (13, 150) and at 86-91% (211), respectively. In the Lithuanian registers the ascertainment was estimated at 100% (10) in the childhood and at 91% (212) in the adult diabetes register.

There was no clear evidence of a decrease in the level of ascertainment for type 1 diabetes cases over time in the Swedish adult diabetes register (DISS) (II). The level of ascertainment during 1992-1997 in the Northern Sweden was similar as during 1983-1987 in the Southern Sweden. However, it was ∼10% lower during 1992-1997 compared to 1986-1991 in the Northern Sweden. Thus, although it is not likely that the tendency of a decrease in incidence seen in the age groups above the age of 14 years should be the result of the differences in the case ascertainment only, our results should be interpreted with some caution (II).

Until 1989 there was no fully independent second source for the childhood diabetes register in Lithuania, and after 1989, although the sources were independent, the primary source was a subset of the secondary source. However, due to the strict organisation of care for children with diabetes in Lithuania the ascertainment should be complete. The main secondary source of the Lithuanian adult diabetes register is not completely independent either, and the patient lists of the Diabetes Society include mainly patients residing in the largest cities and towns. New possibilities are currently emerging for finding independent case ascertainment sources in Lithuania. It would be valuable to check the ascertainment of both Lithuanian diabetes registers with the computerised database of the Social Insurance Scheme (currently under development) in future. This database will be based on prescriptions of insulin, which is free of charge for diabetic patients. Finding additional ascertainment sources would become especially important in future, as the health care system in Lithuania may gradually become less centralized due to a health care reform.

Classification problems in the older age groups

During the childhood and young adulthood (up to the age of 20-30 years) the absolute majority of diabetes cases have type 1 diabetes, and the clinical
classification is rather straightforward. However, in the older age groups classification based on the clinical impression at the time of diagnosis probably underestimates the true number of type 1 diabetes cases, as it is difficult to distinguish cases with type 2 diabetes and latent autoimmune diabetes in adults (LADA) from their clinical characteristics (40, 234, 235). The proportion of cases with type 2 diabetes increases with age (40, 236), and 20-35% of type 2 diabetes cases in the 25-34 years group may have latent autoimmune diabetes in adults (237). Among the cases, not classified as type 1 diabetes at the time of diagnosis in the DISS, ~30% are ICA-positive (236) and ~50% are positive for at least one auto-antibody (either ICA, GAD or IA-2A) (235). Most of these cases probably have type 1 diabetes, as 98% of the ICA-positive and 93% of those positive for any auto-antibody are treated with insulin six (238) and three (235) years after the diagnosis, respectively. Of the cases registered in the DISS ~75% have clear clinical characteristics of type 1 diabetes, thus 30-50% of the remaining cases are potentially misclassified, which would give a 7.5-12.5% higher incidence of type 1 diabetes in the 15-34 years age group in Sweden (paper II). The impact of the misclassification would, however, differ between the age groups, as the proportion of cases, not classified as type 1 diabetes increases with age, being 8%, 20%, 29% and 42% in the 15-19, 20-24, 25-29 and 30-34 years age groups, respectively (236). In the DISS the proportion of cases classified as type 1 diabetes by the reporting physicians did not differ during the four 4-year periods of the study: 71.6%, 73.9%, 74.9% and 74.6%, respectively during 1983-1986, 1987-1990, 1991-1994 and 1995-1998. The corresponding figures for cases that could not be classified as either type 1 or type 2 diabetes at the time of diagnosis was 6.9%, 10.7%, 8.9% and 7.6%, respectively. The proportions of type 1, type 2 and unclassified diabetes cases over time were similar in males and females. Similar classification based on clinical characteristics was used during the whole study period in Sweden, and it seems that there has been no major shift in the classification of the type of diabetes over time. Thus, the estimated incidence change over time in the age group 15-34 years in Sweden should not be seriously biased (II). However, it should be kept in mind that the level of ascertainment for type 2 diabetes in the DISS is low (estimated 53% during 1983-1987) (211), which makes it difficult to assess the true proportion of cases with LADA in the 15-34 years age group.

No antibody testing or C-peptide measurements have been performed in Lithuania (III). However, a study in the DISS material, using similar clinical classification criteria of diabetes in the 15-34 years group, found that only 3 percent of cases treated with insulin since the diagnosis were not on insulin 2.5-3 years later (40), indicating that diabetes was insulin-dependent. Classification of the place of residence after the age of 18 years becomes less
precise (III). A considerable proportion of young people from small towns and rural areas move to the cities for studies, and most often do not return. Thus, a proportion of the cases registered as residing in the five largest cities would have changed their place of residence rather recently. These possible shortcomings might somewhat affect the results of incidence variability by age, and the incidence differences by the urban-rural setting in the older age groups (paper III). It is, however, not likely that such possible misclassification would differ to a large extent between males and females, and between the urban and rural areas in the younger age group to explain the differences found.

Method problems in the case-control studies (IV-V)

Validity of the case-control studies (reduction of systematic error)

Design. Population-based case-control design was used for investigating the association between different environmental factors and the risk of childhood-onset type 1 diabetes (IV and V). Diabetic children were identified from the prospective-nationwide Lithuanian diabetes register (IV), or at four paediatric departments where all children with suspected diabetes from Stockholm area are referred (V). Thus, selection bias should be minimal in both studies. The study base was well defined, and control children were randomly chosen from the population generating the cases – five largest cities in Lithuania or Stockholm area. Thus, control groups should provide a reasonable estimate of the distribution of the exposure in the source population.

Missing information. The level of participation was high in both studies. In the Lithuanian study 94% of cases and 73% of controls returned the filled-in questionnaires (IV). In the Swedish study – 99% of cases and 90% of controls agreed for the dietary interview (V). In the Lithuanian study, however, only 73% of the control families responded and no information about the social class of the non-responders was available. The proportions of responding and non-responding control children did not differ, however, according to the age group and sex. The main reason of non-response among the controls was that they could not be traced (14.5%), mainly because there were no telephones in their apartments. This indicates a lower social status, which in turn is likely to be associated with exposure to more infections. Thus, our results probably underestimate rather than overestimate the association between the exposure to infections and diabetes risk. The majority (over 90%) of the responders had information on the studied exposures.
In the Swedish study 75 (76%) cases and 144 (80%) controls had growth data during the last four years before the diagnosis/interview available, with 2.3 and 2.5 measurements on average, respectively (p=0.22). Analyses of energy intake and relative body weight could be performed in 67 out of 98 data sets (68%). The dietary information did not differ significantly between the cases or controls with and without growth data, the result should not be biased. Further, dietary data for one of the controls were missing in 20 matched sets. In the extreme situation the missing controls may have had a distribution of the exposure close to that of the cases. However, when sucrose and energy distribution of cases was randomly ascribed to the missing controls, the odds ratios were lower but still statistically significant.

Matching. In order to increase the efficiency of the study, matching was used in both case-control studies. In the Lithuanian study (IV) control group was matched for age (birth-year range) and sex at the group level, while in the Swedish study (V) controls were individually matched for the date-of-birth, sex and geographical area within Stockholm. The analyses were performed accordingly – unconditional (IV) and conditional (V) logistic regression. In the Lithuanian study (IV) cases and controls were matched for the year-of birth on the group level, but no matching was done for the month of birth, although month (and season) of birth may be expected to correlate to the frequency of exposure to infections during the first six months of life in the source population. This was because matching for a factor correlated with the exposure in the source population would distort the frequency of the exposure in the control group in the direction of similarity to that of the cases. Such overmatching would bias the estimate toward the null value (no association between the exposure and disease risk). There was a tendency of fewer cases born during the winter months (December-February) compared to the controls, although the overall case-control differences according to the season of birth were not significant (p=0.46). Neither were cases and controls matched for the paediatric district. On one hand, matching for the district could have reduced the possibility of differences in the precision of data recording in the health care booklets (especially infant nutrition). On the other hand, it could have led to overmatching, as children from the same paediatric district would live in close proximity and their exposure to infections could be more similar.

Confounding. Multivariate analyses were used to control for relevant confounding factors.

Sources of information and measurement of exposures
Both studies used prospectively recorded information on the exposures of the study participants. Such information is the most reliable in the epidemiological
studies as it should be free from the disease dependent bias. Still, health care booklet records probably underestimate the true frequency of infections, and completeness of the records about infectious diseases is also likely to decrease when children get older (IV). The duration of breast-feeding estimated from the records is probably also imprecise (may be under- or overestimated). However, it is unlikely that record precision would systematically differ between diabetic and control children, and non-differential misclassification can only lead toward the null value (of no relation). We have adjusted for the duration of mother’s education and birth order of the child – factors which could influence mother’s decisions of consulting a physician for childhood infections, as well as the duration of breast feeding – but the estimates did not change substantially.

To represent the relative body size of the individual (V) we included data recorded during a four-year period before the diagnosis or interview. This was done in order to decrease the impact of possible measurement errors, and to make the number of measurements included comparable for children of different age, as most of the growth data available covered the school age – only 13 (13%) cases and 17 (12%) controls had growth data from the preschool age (0-5 years). Measurements taken during the last three months preceding the diagnosis were excluded, as growth of the case children may be influenced by the metabolic disturbances close to the time of diagnosis.

Dietary history method faces specific difficulties in childhood. It may be difficult for the parent to estimate the dietary intake of the child, as she/he is eating not only at home but also at school or day-care centre, and younger children have a limited ability to cooperate in the interview themselves. The development of the current questionnaire was based on previous experience, and a validation study showed that the method was reliable in school-children (239). The reliability of retrospective evaluation of diet one year earlier is not known. However, both cases and controls would face the same difficulty of recalling their dietary intake, thus any non-differential misclassification would only reduce the strength of the observed associations.

The same dietician interviewed cases and matched controls in order to avoid an interviewer bias, leading to differential misclassification of dietary intake linked to one group of the participants. Much emphasis was laid in the training of the interviewers upon the need to avoid a biased way of asking questions. An interviewer bias resulting in a differential misclassification would probably primarily be reflected by a higher number of food items listed or by reported larger portion sizes. The differences observed between the cases and controls were, however, mainly a result of differences in the selection of foods, and not in the number of food items or portion sizes.
To minimise the possibility of disease-dependent recall bias, and help remember the situation one year earlier, the interview started by mapping of general food habits and meal patterns. A system of crosschecking to the meal pattern and number of breakfasts, lunches and dinners per week, as well as menus from school restaurants was used throughout the interview. Still, some caution is needed in the interpretation of the association between the high intake of specifically disaccharides and sucrose and increased diabetes risk, as the possibility of differential misclassification cannot be excluded completely. The families of case children were interviewed shortly after receiving dietary instructions in association with diabetes diagnosis, that may have influenced their recall of the dietary intake of “harmful” foods containing rapidly absorbed sugars before the diagnosis. It is also possible that our findings reflect the natural course of diabetes development. It has been shown in the Diabetes Prevention Trial that hypoglycaemia may occur in the pre-diabetic individuals during the last few years before the diabetes diagnosis (240). Also in our study irregular insulin secretion and hypoglycaemic episodes during the last year before the diabetes diagnosis could have led to a higher intake of specifically sucrose and other sources of disaccharides. Thus, it is not clear to what extent this result is representative of a longer-term intake.

**External validity (generalisation)**

The biological effect of the studied exposures should be valid for children in general. Diet may play different roles in different age groups (193), thus the selected age interval limits the possibility to draw inferences for the risk of diabetes outside the age of 7-14 years (V).

**Precision (reduction of random error or statistical power)**

Considering the proportions of exposed cases and controls, the Lithuanian case-control study was underpowered for detection of differences in exposure to early infections of the observed magnitude between the cases and controls. This is especially true for the analyses performed in the age group 0-4 years, where only large differences would have been detected. However, for practical reasons of data collection it was not possible to extend either the study period or the study base.
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APPENDIX

Questionnaire (Paper V)