STUDIES ON CHILDHOOD DIABETES MELLITUS.

STUDIES ON CHILDHOOD DIABETES MELLITUS.
(STUDIES OVER DIABETES MELLITUS BIJ KINDEREN)

PROEFSCHRIFT

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Aan Wanda, Iris en Hilgo.

CONTENTS PAGE

Introduction

This thesis consists of a number of collaborative studies aimed at the improvement of the diagnosis and care of children with diabetes mellitus. For the reader, who is not familiar with medical problems, a brief account is given of the clinical "behavior" of the disease (1). It is perhaps clarifying to describe a disease as an entity which may display a behavior as if it were a living being. For non-medical people it sheds some light on the magic doctors seem to operate with.
The many variables by which diseases may manifest their behavior imply that doctors, in caring for patients, constantly perform experiments. During a single week of active practice with a complex disease as diabetes, the clinician conducts more experiments than most of his laboratory collegues do in a year.
The urge of some adolescents with diabetes mellitus to perform even more experiments at home, without their doctors knowing it, generates an even more lively behavior of this disease.
In a psychologic or social sense, the word "behavior" refers to a person's attitude in various situations in life. In a more general sense, the concept of behavior applies to the interaction of an entity to its environment. With this concept, we can contemplate the behavior of a disease during its interaction with the human host (patient), who provides the environment in which the disease conducts its "life".
The disease can behave morphologically, to alter structures in the host's body. It can behave biochemically or biophysically to affect functions of the body. It behaves clinically, to produce symptoms typical for the host's illness, and psychologically as the host will counteract the impact the disease will exert on the host's life. This classification allows a summary of basic problems of childhood diabetes.

Morphologically, the disease is characterized by the destruction of specialized cells, producing a crucial hormone for the metabolism of energy sources and other nutrients, insulin (2). The host is unable to repair this damage and will need insulin substitu-

tion (injections) for the rest of her or his life. The mechanisms leading to this destruction are presently inaccessible for preventive intervention.

Biochemically, failure to completely normalize the metabolism of energy sources and other nutrients is a hallmark of the disease, despite the availability of highly purified insulin preparations. A spectre of chronic metabolic disregulations is still found, often with remarkably few symptoms (3).

After 10 or 20 years the average patient will display an array of structural and functional abnormalities. These are called the chronic complications of diabetes. They have shortened the life expectancy of children with diabetes mellitus by 1/3 in years.

Clinically, there are presently no means to prevent or even stop the irreversible damage to cells producing insulin.

A classic controversy in medicine (4) has been whether meticulous insulin-administration and diet-keeping could alter the behavior of the disease in its tendency to develop the incapacitating complications. One of the reasons for this controversy is the lack of practical biochemical parameters, to monitor the quality of the metabolic regulation as a result of such therapeutic regimens (5).

Psychologically, the child has to grow up with one or two daily shots of insulin, dietary measures, to experience regular hospital-visits, including admissions for unforeseen severe disregulation of the diabetes (6).

The parents are usually aware of the possibility of chronic complications and have to live with the idea their child's inconspicuous disease is at least partially inherited. The uncertainty whether strict following of medical desirabilities, interfering with the child's upbringing, would improve the long-term prognosis or not, is an embarrassment to health-care professionals as well as patients.

The ramifications of these problems of childhood diabetes provide a daunting prospect, if the behavior of this disease cannot be modified.

Although the understanding of the disease is far from complete,

advances in knowledge have been made steadily since the discovery of insulin in 1921. In the pre-insulin-era diabetes mellitus in children meant "melting" away in a couple of weeks or months with certain death. The impact of the discovery of Banting and Best, that their pancreatic extract saved the lives of these children since 1922 was great. The dramatic effect of injected insulin on the blood glucose of patients with hyperglycemia undoubtedly led to the belief that hyperglycemia, and hence diabetes mellitus, was always due to insulin lack, and the more profound the insulin deficiency, the higher the glucose concentration. It was not until 1959 that the painstaking studies of Somogyi demonstrated that excess insulin could in fact exacerbate the diabetic state (7). Just thereafter, in 1960, Berson and Yalow published their historic finding that insulin-concentrations could be measured in biologic fluids by radio-immuno-assay. This finding was preceded by their observation that many patients with diabetes had antibodies against the injected insulin in their circulation (8).

Early in the 1930's blindness caused by longstanding diabetes, treated with insulin, was virtually unheard of. Now it is appreciated that diabetes is the leading cause of new cases of blindness (9). The rediscovery of an old medical file in the cellars of the Hospital for Sick Children in Toronto exemplifies this point: it contained an autopsy performed in 1932 on a man who had been one of the very first patients ever to receive insulin. A full description of abnormalities classic for chronic complications was in the report, but it was stated publicly the young man had died from a motor-cycle accident (10).

The past decade has delivered an explosion of data on insulin dependent diabetes, as a comparison between the proceedings of large meetings of 10 years ago and today would demonstrate: immunology, virology, epidemiology (11), pharmacokinetics (12) and biochemistry (13) have made steady contributions.

With the echo of these explosions of knowledge ringing in the ears, the clinician selects those topics that would benefit the patient most, either directly or indirectly.

This thesis is the result of the selection and subsequent work on such topics between 1978 and 1984.

It consists of three chapters, each divided into introductory paragraphs, a selection of papers relevant to that chapter and a discussion of its contents, concluded with a summary. Figures and tables are numbered by their sequence throughout each chapter, excepting illustrations of the reprints of papers. Also, the literature references cited throughout each chapter are only given at the end of that chapter and do not refer to literature references given in the reprints of papers.

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CHAPTER I ETIOLOGY

For I dipt into the future,
far as human eye could see,
Saw the vision of the world,
and all the wonder that would be.

Alfred, Lord Tennyson, 1809-1892.
(From: Locksley Hall 1.119)

§1. Background

The recognition of thirst and honey-sweet urine in massive amounts as signs of the disease is old. In ancient India descriptions have been found in texts on Hindu medicine (1). Diabetes mellitus as a term is derived from the greek diabainein (to fall through) and mellos (honey-sweet) (2).
Today the notion that a variety of genetic and evironmental factors may lead to diabetic states and sequelae thereof, prompted attempts to associate these factors with clinical classifications. However, etiologic factors may be expressed in several clinical forms and putative clinical presentations may involve several etiologic factors. Associations are only valid in a "statistical" manner. What has been learned from such associations?
A first fundamental point is that insulin-requiring or insulin-dependent diabetes mellitus (also known as juvenile-onset, ketosis prone or labile diabetes) is a distinct disorder from non-insulin-requiring diabetes mellitus (maturity-onset, non-ketosis prone or stable diabetes). A new classification separating these broad categories has been put forward (3,4), distinguishing type-1-diabetes (insulin-dependent diabetes mellitus - IDDM) from type-2-diabetes (non-insulin-dependent diabetes mellitus - NIDDM). This classification has subsequently be refined (5).

Table 1. Classification of diabetes mellitus (5)

Type 1	- insulin-dependent diabetes - genetic susceptibility known
1.a.	- predominantly young onset - slight male excess - transient autoimmune phenomena - probably initiated by viruses - primary association with both HLA*-DR3 and HLA-DR4
1.b.	- predominantly middle age onset - often insiduous - striking female preponderance - tendency for persistent autoimmune phenomena - strong family history for autoimmune disease - strong association with HLA-DR3
Type 2	- non-insulin-dependent diabetes - genetic susceptibility unknown - strong familial segregation
2.a.	- associated with obesity - inheritance of inappropriate metabolic genotype? - abnormality of centrally mediated control of blood glucose?
2.b.	- abnormalities of insulin receptor concentration and affinity on target cells?

* HLA - human leukocyte antigens.

The relevance of this later classification is, that it distinguishes childhood diabetes mellitus in its clinically classic form as type-1a-diabetes from other diabetic syndromes with insulin dependency. Diabetes mellitus other than type-1a is not the

subject of this thesis. There are many unusual, but genetically well characterized, childhood syndromes associated with glucose intolerance, such as dystrophia myotonica, Werner's syndrome, Down's syndrome and lipodystrophies. These syndromes, together with the less rare maturity-onset-type-diabetes of the young and diabetes associated with cystic fibrosis, have been reviewed by Rimoin (6). These unusual syndromes comprise less than 5 percent of all diabetic syndromes of childhood, 95 percent or more of the children using insulin are considered to have type-1a-diabetes mellitus. None of the patients described in this chapter had any of these syndromes, except the patient reported in the second paper. Therefore all others are considered to have type-1a-diabetes.

The characteristics of type-1a-diabetes indicate that the disease is possibly related to an environmentally initiated, perhaps viral (7) perturbation, that causes destruction of the child's own pancreatic beta-cells (8), which normally produce insulin.

A second fundamental point raised in table 1 is that type-1-diabetes per se is not inherited, but it is the susceptibility to develop the disease which is transmitted (9). This susceptibility is associated with genes of the major histocomptability complex, in particular expressed by HLA-DR3 and HLA-DR4. It is unknown how these genes cause this susceptibility.

Table 1 also indicates that the disease is characterized by transient autoimmune phenomena as a reflection of such involvement of the pancreatic beta-cells. These autoimmune phenomena, albeit transient, were found to be relatively common in adult patients (10). Irvine then developed the following model of the etiology of type-1-diabetes, visualized in 1978.

Fig. 1

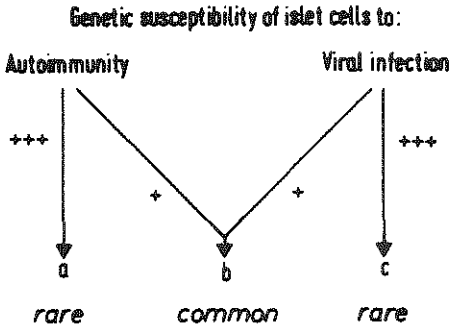

Fig. 1. The possible interaction between autoimmunity and pancreatotropic viral infection in the pathogenesis of type-1-diabetes, based on genetic susceptibility to either or to both. Source: Irvine, 1978, Lancet, i, 638. Reprinted with permission.

The ultimate goal of any study into the etiology of chronic or irreparable disease is its prevention. The Irvine-model combined the features related to the etiology of childhood diabetes effectively. It should be noted that Irvine already suspected that acute viral infections rarely caused insulin dependent diabetes. The primary goal of the studies of this chapter was to identify children at risk for the development of insulin dependency, paving the way for the consideration of future (immuno-)preventive measures.

What sort of preventive measures might be considered is discussed at the end of this chapter (paragraph 5).

The basis for the studies reprinted in this chapter was the following interpretation of the Irvine-model of the natural course of diabetes type-1a: As the nature of the viruses and hence their mode of action is unknown (Chapter II - paragraph 3), interactions of viral infections with autoimmunity leading to pancreatic beta-cell destruction may be gradual and repetitive. The disease process may evolve insidiously over years in contrast to the im-

pression the clinician obtains from a history of some weeks of diabetic symptoms on admission of a child to the hospital for initial treatment.

The grounds for the idea, that the disease process of childhood diabetes might be insidious and asymptomatic, were found in the literature on the pathology and the dynamics of insulin secretion, summarized in the next paragraph.

§2. Pathology and insulin secretion

The hallmark of the pathology of childhood diabetes is a selective loss of beta-cells of the islets of Langerhans normally producing insulin (11).
What loss of beta-cells can be sustained without deterioration of glucose homeostasis? Or, conversely, what is the minimum amount of beta-cells necessary to prevent diabetic symptoms?
The studies by Martin and Lacy in 1963, using experimental surgery, indicated that diabetic symptoms do not develop as long as 20 to 30 percent of the pancreas remains *in situ* (12). They also found that a decrease in total insulin reserve required a parallel decrease in body weight to maintain glucose homeostasis. This explains the clinical experience that weight loss may precede marked polyuria and polydipsia in children developing diabetic symptoms over weeks or months. In children where this sequence of events can be documented using previous weights and heights, a gradual loss of pancreatic beta-cells is likely, whereas almost simultaneous loss of weight with the advent of diabetic symptoms would suggest a more acute loss of beta-cells.
Accordingly, injection of mice with compounds specifically toxic to beta-cells, such as streptozotocin, produce graded losses of beta-cells. Those studies too revealed that fasting hyperglycemia can be prevented until 70-80 percent of the islet-cell mass is lost (13).
The studies by Martin and Lacy (12) indicated in addition that remaining beta-cells after major losses may become functionally more active, leading to increased synthesis and release of insulin compared to normal beta-cells. That finding is in keeping with clinical studies in humans indicating that many newly diagnosed children (and adults) have some residual beta-cell-function and that improvement of the metabolic state will enhance the remnant capacity of the diabetic pancreas to secrete insulin (14, 15).
An important question then becomes, to what extent beta-cells have the ability to regenerate and recover from initial lesions. This is essentially unknown. By radio-labelling of islet-cells,

including beta-cells, in vivo in animals and in vitro in tissue-culture, a slow turn-over of intact new islet-cells replacing old islets has been documented. It is now generally accepted that islet-cells may regenerate, but whether new beta-cells can be formed once they are destructed, is unknown (16). To answer this question in human patients, serial pancreatic biopsies would be required, which is obviously ethically prohibited. Also such biopsies may well show different results, depending on the part of the pancreas they were taken from (17).

The islets of Langerhans encomprise about 2 percent of the total weight of the pancreas and consist of 65 percent beta-cells, producing insulin, C-peptide and pro-insulin, 17 percent alpha-cells, producing glucagon, 9 percent delta-cells, containing somatostatin, 9 percent P.P.-cells, producing pancreatic polypeptide (P.P.) (16). In childhood diabetes, the loss of endocrine cells is highly selective for beta-cells, whereas alpha-cells, delta-cells and P.P.-cells initially remain in amounts similar to or higher than those in the normal pancreas. From the few pancreatic specimens available from deceased recently diagnosed diabetic patients, it appeared some beta-cells remained and some even proliferated (11). Ultimately, however, all endocrine pancreatic tissue is lost and replaced by fibrous tissue. The apparently temporal proliferation was erratic and had little resemblance to the normal architecture of islets of Langerhans: few small strands along pancreatic ductuli were found, consisting of mainly P.P.-cells. This very partial regeneration does not remind of stages of the embryologic development of islets, as one might think.

During normal human embryologic development, the first islet-cell to be identified is the more numerous alpha-cell (A2) at 9 weeks, shortly followed, at 10½ weeks by beta-, delta- (A1) and P.P.-cells (16). The alpha- and delta-cells continue to be more numerous than beta-cells throughout fetal life. In the rat, rapid expansion of beta-cells takes place about mid-gestational, whether or when this occurs in humans is unknown (18).

An important pathologic hallmark of childhood diabetes, was the old finding of mononuclear cell infiltrates around the islets of

diabetic children (19), designated as "insulitis", later confirmed by Gepts and his associates (20).
About two-thirds of the pancreases of children dying following the acute onset of diabetes will have infiltration of granulocytes and monocytes, in particular around the islets. Similar changes are almost never seen in adult diabetics (20). A few cases of insulitis with an infiltrate consisting of lymphocytes have been reported in non-diabetics, all in very young children (18).
About one-third of the pancreases of diabetic children examined shortly after diagnosis showed "hydropic degeneration" accompanied by lymphocytic infiltration. This picture suggests a more chronic inflammation (20).
Acute inflammation, involving other polymorphonuclear cells, has also been noted in diabetic children expiring very shortly after an acute onset of diabetes. By contrast, insulitis of the newborn child of a diabetic mother will usually show a predominance of eosinophils (16). The rather constant finding of lymphocytes in and around islets of diabetic children, evidence for cell-mediated autoimmune mechanisms, the association with other autoimmune endocrinopathies and the presence of auto-antibodies against the islets of Langerhans has led to the "autoimmune hypothesis" of childhood diabetes (figure 1). Whether such involvement is reversible or whether regeneration of islets before the stage of clinically manifest diabetes may occur is unknown.
Can the pre-diabetic state of childhood diabetes be detected by partially impaired insulin secretion? There is a lack of long-term studies of cohorts of children with such impairment, in the absence of overt diabetic symptoms. Furthermore there are few data on standardized insulin responses after oral glucose loading with the exception of the classic Rosenbloom-studies (21). Their group traced 105 of 140 siblings of children with insulin-dependent diabetes, who had had an oral glucose tolerance test 10-12 years earlier. Six of these 105 children had developed insulin deficiency in the meantime. Nineteen of these 105 children had shown 7.8 mMol/L glucose or more, 2 hours after glucose loading orally. Five of the six siblings that later developed diabetes,

belonged to those 19 children (22). Obviously such data may not be reversed: it has been estimated only 0-10 percent of children with such defined "chemical diabetes" do develop diabetes (23). The simultaneous measurement of insulin or 4 more glucose-values up to 4 hours after the ingestion of glucose did not improve the predictability of these "prediabetic" siblings. The data implied poor sensitivity and poor predictability of oral glucose tolerance tests to foretell clinically overt diabetes mellitus, but suggested a long latency of diabetic symptoms in some cases.

The (remnant) endogenous capacity of the pancreatic beta-cells to secrete insulin may be estimated in patients already treated with insulin by the measurement of "connecting-peptide" (C-peptide). Insulin is formed from pro-insulin, which consists of insulin and C-peptide. C-peptide is secreted in equimolar amounts to insulin by the pancreatic beta-cells into the circulation after cleavage of the pro-insulin. C-peptide will not react significantly with circulating (exogenous) insulin in suitable radioimmunoassays and may thus be measured separately. This is mostly done after administering one of several suitable stimuli, so that the maximal (remnant) capacity of the pancreatic beta-cell to endogenously secrete insulin (as C-peptide) is assessed (24).

Partial, temporary, remissions of endogenous insulin secretion are frequently seen in recently diagnosed children. This episode is called the "temporary remission phase" or the "honeymoon-phase" during which low doses or even no insulin substitution may be necessary over weeks or months after the initial treatment, requiring higher dosages again thereafter (23). This apparent temporary recovery of the function of pancreatic beta-cells may be documented by the measurement of C-peptide, despite the circulation of exogenous insulin (14).

Taken together, the literature data suggest that clinical symptoms may not develop until some 70-80 percent of endocrine tissue of the pancreas is lost. This destruction is accompanied by mononuclear cell infiltrates and selective loss of the pancreatic beta-cells, without signs of organized regeneration of the islets of Langerhans.

Insidious deterioration of glucose-tolerance in sibs of diabetic

children, that later developed the disease themselves, did occur, but this testing had poor sensitivity in predicting such cases. None of these data spoke against a symptom-free, perhaps years long, prediabetic phase in diabetes-type-1a.

§3. Scope of the studies

When the studies reprinted in paragraph 4 were initiated, the association of the susceptibility to the disease and genes of the major histocompatibility complex (MHC) appeared the only tangible marker. Therefore a detailed analysis of these genes was made, described in the first paper of this chapter. The relation between HLA-antigens and disease in general is reviewed in the thesis of B.M. de Jongh (Leiden, 1983).

It had been found that the relationship between two specific HLA-antigens (HLA-DR3 and HLA-DR4) and insulin dependency was particularly strong: some 90 percent of children with diabetes-type-1a had either of these haplotypes (5). Thus, the absence of either haplotype in children with proven total insulin-deficiency may point to unusual pathogenetic mechanisms leading to the disease. This application is exemplified in the case report given in the second paper of this chapter.

Another purpose of the study on the genetics of HLA in diabetes was to answer the question to what extend HLA-identity carries a risk to siblings of diabetic children. In other words: what are the chances of a child that is HLA-identical to a diabetic sib to become diabetic itself over a given period of time, versus the chances of a sibling that is only haplo-HLA-identical or non-HLA-identical? From the data in the reprint of paper 1 the recurrence risk for HLA-identical siblings was calculated at 11.6 percent. Thus, only 1 in 8 HLA-identical siblings would actually develop the disease.

This was a figure of little predictive value if children at risk were to be identified, with the consideration of preventive measures in mind. Therefore additional associated factors were examined: the transient autoimmune phenomena.

The various methods for the detection of such islet-cell antibodies, lacked a definition of their accuracy (25).

This premise was confirmed in 1981 by comparative studies using fresh frozen sections of pancreatic tissue: assay precision and reproducibility of islet-cell cytoplasmatic fluorescence were such, that a multicentre study revealed only 50 to 60 percent

concordant scores of diabetic sera between four different laboratories (A. Drash, 1981, at the annual meeting of the International Study Group of Diabetes in Youth, Les Collons, Switzerland). This was not surprising as the control of technical details, such as the quality of fluorescent antibodies, microscope lamp and filters, was not previously established and the expression of results in titres rather than "scores" was exception rather than rule. A comparison of methods had to be made for this particular method.

In a collaborative study we examined (26) the sensitivity and specificity of the assay for islet cell cytoplasmatic antibodies in human serum using cryostat sections from freshly frozen pancreas. The specificity of the assay was close to 100% while the sensitivity was 40%-98% depending on the pancreas used. Inter-observer variation was 12-27%. End-point titres of islet-cell antibodies varied with the sensitivity of each pancreas. End-point titration of the antibodies in two different laboratories using the same pancreas was significantly correlated (Spearman's test $p < 0.001$). We concluded that a reliable determination of islet-cell antibody titres in human serum requires careful characterization of the sensitivity and specificity of each pancreas used as a source of frozen sections, in the indirect immunofluorescence assay. Given these findings we selected one of the particularly suitable pancreatic specimens (designated "HP" - kindly provided by the Department of Surgery of the Dijkzigt Hospital, Rotterdam) for further studies.

The rationale for the study of islet-cell antibodies was that these were seen at diagnosis, but disappeared thereafter in a few years (27). If one is seeking a marker of children at risk for the development of diabetes, it is conceivable that the islet-cell antibodies might be present before the disease becomes actually manifest.

Given an assay-system with known sensitivity and precision for the detection of islet-cell antibodies, the accuracy of the prediction of childhood diabetes was explored retrospectively, described in the third paper of this chapter.

In the meantime the late dr. Andrew G. Cudworth and his collabo-

rators in London reported that one particular property of islet-cell cytoplasmatic antibodies, complement fixation, appeared to be present in circulating antibodies of children that later developed diabetes. It was suggested that this particular property was associated with pre-diabetic stages, as it disappeared rapidly after diagnosis (28).

Subclasses of IgG fix complement in the order $IgG_3 > IgG_1 > IgG_2 > IgG_4$.

This suggested that subclasses of IgG - namely IgG_1 and IgG_3 - might be preferentially involved in the children's autoimmunity towards islet-cells before the disease becomes clinically manifest. Restrictions to certain subclasses of IgG had been found in the following diseases: two cases of myasthenia gravis had exclusively IgG_3 antibodies to the acetylcholine-receptor; complement fixing antibodies to epidermal basement membrane in herpes gestationis were mainly IgG_1; in bullous pemphigoid non-complement fixing antibodies contained only IgG_4 (29). Also preferentially IgG_3 was seen in human viral antibody activity (30). As the IgG-subclasses by which children's auto-antibodies reacted were unknown, the above suggestion of possible restriction of the IgG-subclasses involved was important for two reasons:

1. IgG-subclasses are isotypes of the heavy chains of immunoglobulins that are coded for by chromosome number 14. The isotypes (IgG_1, IgG_2, IgG_3, IgG_4) coded for by the Gm-locus comprise different allotypes. Restriction to certain IgG-subclasses might suggest the involvement of non-HLA-related genes, which explain the inherited susceptibility only partially. Thus the finding of additional susceptibility-genes for the detection of children at risk might have considerable importance. Even more so, since Gm-genes were demonstrated to be involved in the susceptibility to Graves disease, next to HLA-linked genes (31).

2. The isolation of IgG-subclasses specifically involved in islet-cell autoimmunity might be used to detect subcellular fractions of islet-cells serving as antigens to these auto-antibodies.

The complement-fixation and IgG-subclasses of children's islet-

cell antibodies were therefore examined. This is described in the fourth paper of this chapter.
So far the studies were aimed at the identification of children in prediabetic-stages, for which stages future techniques for intervention might be considered, that might stall ongoing pancreatic beta-cell destruction.
It should be mentioned here that the children and their parents participating in the investigations into prediabetic stages gave their consent if these consisted of single blood-drawings at home, which was done outside working hours. It was agreed upon beforehand the results of the measurements would not be disclosed to them.
At this point in our studies, we turned to another stage in the course of childhood diabetes, namely the first year of treatment of the manifest disease.
After initial treatment, about 2/3 of the children experience a temporary remission in their insulin requirements, also named the honeymoon-phase, as explained in paragraph 2. This 50 percent or larger reduction in insulin dosages, coincides with an apparent recuperation of the endogenous insulin secretion (32). However, the reason for this phenomenon has never been fully elucidated. If this partial regeneration of the capacity to secrete insulin could be maintained, this might have important consequences for the children. It had been suggested type-1-diabetic patients with even remnant capacity to secrete insulin had less retinopathy after 15 years of disease-treatment (33), but this important association has been questioned by others (34). Also, even partial endogenous insulin secretion protected against the formation of ketone bodies, when insulin treatment was withdrawn (35).
During a retreat of the Department of Immunohematology of Leiden University aboard the "Eendracht", this curious remission in insulin requirements after the initiation of insulin injections was discussed.
With regard to this phenomenon, Prof. I.R. Cohen of the department of Cell Biology of the Weizmann Institute referred to their finding (36) of the generation of insulin-anti-idiotypes in mice as a consequence of the vaccination with insulin. These anti-

idiotypes were probably antibodies to the insulin-antibodies as a part of an idiotype-anti-idiotype regulatory network, put forward by Prof. N.K. Jerne in 1974 (37). Prof. Cohen supposed that such idiotypic antibodies might also be raised in children by the inevitable insulin-substitution.
Further it was reasoned that some of these anti-idiotypes might be internal-image-specific, meaning they would "mirror" the structure of the original antigen, insulin. In other words: if "classic" antibodies to insulin were raised, the anti-idiotypes raised against these "classic" antibodies might in turn have the structure of the antigen they were originally derived from. Such anti-idiotypes, depending on the specific quantities that emerged, might be expected to have effects at the level of the insulin receptor (36).
The nature of insulin receptor antibodies found in children is described in the fifth paper of this chapter.
The bearings of the findings, reprinted as papers in the next paragraph, to the consideration of possible preventive intervention will be discussed in paragraph 5.

§4. Addendum

Paper 1

HLA and GM in insulin-dependent diabetes in the Netherlands: report on a combined multiplex family and population study

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ABBREVIATIONS from: AR = Autosomal recessive; EF = Etiologic fraction; GM = Gamma markers, allotypes of immunoglobulin G heavy chain; HLA = The MHC of man; HLA-A,-B,-C-D/DR = Loci of the HLA system; H-W equilibrium = Hardy-Weinberg equilibrium; IDD = Insulin-dependent diabetes mellitus; MHC = Major histocompatibility complex; NIDD = Non-insulin dependent diabetes mellitus; OR = Odd's ratio; RAD = Rare autosomal dominant; RR = Relative Risk

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Summary

This report deals with the genetic factors involved in insulin-dependent diabetes mellitus (IDD) in The Netherlands.
Twenty-two Dutch multiplex families with IDD were typed for HLA-A, -B, -C and -DR antigens, for BF, C2, C4 and GLO polymorphisms as well as for GM allotypes of immunoglobulins. In addition, 53 unrelated IDD children and 31 unrelated patients with adult onset IDD were typed for HLA-A, -B, -C and -DR antigens. A significant heterogeneity for the frequency of HLA-DR4 related to age of onset was observed. A significant deviation of the Hardy-Weinberg equilibrium was observed for the HLA-DR locus with an excess in patients of heterozygotes HLA-DR3, -DR4. HLA-B8 and HLA-B15 were not only secondary associated, but constituted with HLA-DR3 and -DR4, respectively, a haplotype in association with IDD.
Non-random segregation of HLA-haplotypes was observed in multiplex families exemplified by an excess of HLA-identical affected sibpairs. Cross-overs between HLA-DR and GLO identified the HLA-DR segment as mainly involved in the association with IDD. Three diabetic haplotypes were confirmed to occur frequently among affected sibs:
a) A1, B8, BFS, C2.1,C4AQ0,C4B1, DR3,GLO2
b) Aw30,Cw5,B18,BFF1,C2.1,C4A3, C4BQ0,DR3,GLO2
c) A2,Cw3, B15,BFS, C2.1,C4A3, C4B3, DR4,GLO1
The segregation of GM allotypes to affected sibpairs was not significantly different from random segregation.
The main conclusions from this study are that significant heterogeneity for age of onset exists and that the data are not compatible with simple genetic models including dominant, recessive and intermediate models of inheritance. The data do require more complex models, involving two different HLA-linked (sets) of susceptibility genes.

Introduction

The reported association of genes of the Major Histocompatibility Complex (MHC) with insulin-dependent diabetes mellitus (IDD, type 1) and not with non-insulin dependent diabetes mellitus (NIDD, type 2), has enabled considerable progress in studies of the genetics of IDD.

Singal & Blajchman (1) were the first in showing an increase in frequency of HLA-B15 among patients as compared to normal controls. Other investigations confirmed the HLA-B15 association and revealed an association with HLA-B8 as well (2). Later on, the HLA-D and -DR alleles Dw3, Dw4 and DR3, DR4, respectively, were shown to have an even stronger association with IDD. Furthermore, DR2 was significantly decreased among patients (3,4). Studies of families with more than one affected sibling revealed an excess of HLA-identical affected sibpairs (5).

These data indicated that the susceptibility for IDD was related to the HLA system (6) which led to a substantiation of various genetic models for inheritance of IDD. These models encompassed a dominant (7), recessive (8), overdominant (9) and intermediate mode of inheritance (10).

This variety of models can partly be explained by the fact that environmental factors are at least as important as genetic factors in the development of IDD, as may be inferred from the observation that less than 50 percent of monozygotic twins are concordant for the disease (11). Our studies focussed on the genetic factors involved in IDD in The Netherlands. Three aspects will be considered in more detail:

Genetic heterogeneity in IDD, genetic factors of IDD related to the HLA system, and non-HLA genetic factors involved in IDD.

Regarding the first aspect, several factors involved in genetic heterogeneity of IDD have been proposed. An association of IDD with other autoimmune diseases, such as thyroiditis and Addison's disease, is related to HLA-B8, -DR3 with persistance of islet-cell antibodies (ICA), whereas IDD without other autoimmune diseases is characterized by a transient presence of ICA and an association with either HLA-B15, -DR4 or HLA-B8, -DR3 (12). Stu-

dies on genetic heterogeneity associated with the age of onset are conflicting. Sibpair analysis in multiplex families showed a lack of concordance for age of onset (13). However, HLA-DR4 and HLA-B15 are particularly increased in patients with a young age of onset (14,15). Thus it appeared worthwhile to investigate the association of HLA and IDD with regard to age of onset of the disease.

The second aspect refers to the characterization of HLA related markers associated with IDD. To this purpose we investigated 75 unrelated IDD patients with onset of the disease before the age of 17. Among the different tests, to detect associations, we employed a recently described method to detect haplotype associations (16). In addition, 22 multiplex families were genotyped for HLA-A, -B, -C, -DR, complement factors (BF, C2, C4) and GLO, and analysed for HLA haplotype segregation and occurence of specific haplotypes related to IDD. The detection of HLA-DR-GLO crossovers and a clustering of "diabetic" haplotypes allowed an analysis of the chromosomal segments associated with the susceptibility for IDD.

Regarding the third aspect, we investigated whether non-HLA genes are involved in IDD. This question was prompted by the observation of a discrepance in concordance rate for IDD of 50 percent among monozygotic twins (11), as compared to 12-14 percent in HLA-identical affected siblings (17,18).

Gm, the allotype of immunoglobulin heavy chains was studied because this marker was reported to be associated with other HLA-related immunopathogenic diseases (19,20).

Subjects and methods

I. Subjects

All patients included in this study were diagnosed as having insulin-dependent diabetes, according to criteria of the National Diabetes Data Group (21) and were of Dutch Caucasoid extraction.

A. Unrelated patients

The unrelated patients were divided into three groups:

1. Unrelated patients with age of onset of IDD after 17 years (n = 31).
2. Unrelated patients with age of onset of IDD before 17 years (n = 53).
3. Propositi of multiplex IDD families with age of onset before 17 years (n = 22).

Group 2 and 3 (n = 75) were combined in the population analysis.

ad. 1 The first group comprised 31 unrelated patients with recently diagnosed IDD and age of onset after 17 years, who consecutively entered the adult-diabetic out-patient clinic of the University Hospital in Leiden. Of 31 patients, nine were female and 22 male (ratio = 2 : 5); the mean age of onset was 30.34 years, ranging from 17.5 to 63.5 years.

ad. 2 Group two comprised 53 unrelated children with IDD and age of onset before 17 years, attending the out-patient clinic for diabetes at the Sophia Children's Hospital in Rotterdam. Of these 53 patients, 24 were female and 29 male (ratio 4 : 5); the mean age of onset was 6.94 years, ranging from 1.25 to 16.75 years.

B. Multiplex families

Of 22 Dutch Caucasoid multiplex families, 19 responded to a call for families with more than one diabetic patient, at a yearly meeting of the Dutch Diabetic Patient Union (DVN). The remaining three families were known at the Sophia Children's Hospital. The families originated mainly from the Western part of the Netherlands. With the exception of one healthy child the families were completely investigated, and genotyped for HLA-A, -B, -C and -DR. In addition, most families were typed for BF, C2, C4, GLO and GM. In 12 of the 22 families, only two children were affected and in six families one parent and two children. In two families, three children and in one familiy, four children were affected.

In one family both members of a monozygotic twin were affected, and this family was not included in the haplotype segregation

analysis. Arbitrarily, in each family the eldest affected child was assigned as the index patient (propositus). In total, we found 22 propositi (12 female, 10 male, mean age of onset 7.0 years, range from 1.3 to 16.8) and 32 secondary patients (12 female, 20 male, mean age of onset 10.6 years, range from 1.3 to 44).

Marker phenotyping

The HLA typing was performed by the standard microcytotoxicity assay, using peripheral blood lymphocytes. 16 HLA-A, 40 HLA-B and 6 HLA-C antigens were determined, using a set of 120 well-defined alloantisera (22). With the two-colour fluorescence assay 10 HLA-DR antigens were investigated, using 80 different alloantisera (23).

Properdin factor B (BF), complement factor 2 (C2), and complement factor 4 (C4A and C4B) were determined by electrophoretic techniques (24,25).

Electrophoretic phenotypes of glyoxalase-1 (GLO1) were determined using cellogel techniques (26).

Allotypes of immunoglobulins (GM, AM and KM) were typed by haemagglutination-inhibition assay (27).

Genetic analysis

As normal controls, a panel of 1018 local blood donors was used for the HLA-A, -B and -C antigens and a panel of 201 randomly selected Dutch Caucasoid individuals for the HLA-DR antigens. The HLA antigen frequencies in patients and controls were compared using the method given by Woolf as modified by Haldane (28). If mentioned, the P values were corrected for the number of comparisons made (29).

Heterogeneity between estimates of relative risks was analysed with a chi-square test (28). Heterogeneity in relative risk for HLA-DR3 and HLA-DR4 was investigated among IDD patients with onset of the disease before the age of eight, between the age of eight and 17, and after the age of 17. The age of 17 was chosen because at that age no overlap occurred between the patients from the adult Leiden clinic and the childhood patients.

As another parameter to determine the strength of an association, the etiologic fraction (EF) (30) was estimated for a selected group of antigen combinations. Generally speaking, the EF value indicates how much of an association is "due to" the disease associated factor, and under certain conditions the EF provides information about the degree of linkage disequilibrium between a marker gene and an assumed disease susceptibility gene. Gene frequencies, used for the Hardy-Weinberg (H-W) expectations were calculated by gene counting in the patients and estimated with the square-root method in the controls. In comparing subgroups of allelic combinations, we employed also the odd's ratio (OR), as defined by Svejgaard and Ryder (31) with the subgroup of phenotype (HLA-non DR3, non-DR4 (= HLA-Drx, x)) as reference.

A striking feature of the HLA system is the degree of linkage disequilibrium among alleles of closely linked loci. For this reason an association with an allele of one locus often leads to a secondary association with an allele of a locus in linkage disequilibrium with the first locus. It is, however, important to distinguish whether an association is secondary, due to linkage disequilibrium with a primarily associated antigen, or whether the association is due to a real haplotype association. In the latter case, a third order linkage disequilibrium is implicated between an assumed disease locus and two test loci. Recently, Porta & McHugh (16) have developed a method to calculate a delta-value for a third order linkage disequilibrium from population data. Two extreme genetic models, rare autosomal dominant (RAD), and autosomal recessive (AR) are investigated, based on the assumption of a disease locus with a normal and a disease allele.

We will refer to the delta-value for a third order linkage disequilibrium as delta-haplotype.

The occurence of HLA-DR and GLO cross-overs has been analysed in affected sibpairs, one of which had a cross-over. The question was addressed whether the sibs shared the HLA-DR, or the GLO segment, thus delineating the "diabetic" segment (32).

The haplotypes, determined in the genotyped families, were divided in three categories, according to a suggestion of J.H. Edwards (personal communication):

1. Haplotypes in patients and shared with the index patients ("hot" diabetic haplotypes), 2. haplotypes in patients but not shared with the index patients ("warm" diabetic haplotypes), and 3. haplotypes only present in normal individuals (non-diabetic haplotypes). The "hot" and "warm" diabetic haplotypes were combined and clustered according to HLA specificity (starting with HLA-DR). Analysis of the clusters allows to characterize among unrelated "diabetic" haplotypes, those segments which are particularly conserved in evolution and associated with susceptibility for diabetes. Since only those haplotypes are selected that are associated with IDD, it is now possible to learn from cross-overs that occurred in history: cross-overs and mutations that do not alter the predisposing capacity of the chromosome are present in the "diabetic" haplotypes and thus may help to delineate the segment where the factor(s) associated with susceptibility for IDD is present.

Non-random segregation of parental haplotypes to the children was analysed with the method of haplotype sharing as given by Thomson & Bodmer (8) and with the method given by De Vries et al. (33). Because the latter may not be generally known, we will describe this method of segregation analysis to affected or normal siblings.

When the paternal haplotypes are a and b, and the maternal haplotypes are c and d, D is the absolute value of the number of sibs with a minus the number with b (or: the number with c minus the number with d). The expected value of D is d, and can be calculated for each sibship-size and so can the variance of d = (σ_d^2). The sum of the D values (ΣD) of the various sibships should be supposed to be equal to the sum of the d values (Σd). When, however, an association or linkage would exist ΣD would have a higher value than that of Σd. The difference between the observed values of ΣD and of Σd can be tested for its statistical significance in the following way:

$$X^2 = \frac{\Sigma D - \Sigma d - 0.5}{\Sigma \sigma d^2}$$

and the value of P, found in a X^2 table, is twice the suitable P value.

Results

We will first describe the results of the analysis of heterogeneity related to age of onset. Secondly, we present the data of a population analysis of patients with age of onset before 17 years and thirdly we report the findings in the multiplex families.

Age of onset

Table 1 shows the relation between the age of onset of IDD and the frequencies of HLA-DR3 and -DR4. Three categories were compared: onset of IDD before the age of eight, between the age of eight and 17, and after 17 respectively.

Table 1. Frequencies of HLA-DR3 and HLA-DR4 in IDD patients, related to age of onset in years.

	Age of onset	HLA-DR3 pos.	HLA-DR3 neg.	Rel.Risk	X^2	X^2 Hetero-geneity	P°
Controls		58	143				
	0- 8	31	18	4.2	18.96		
Patients	8-17	15	11	3.3	8.43	0.20*	NS
	>17	13	18	1.8	2.29	2.87**	NS
		HLA-DR4					
Controls		49	152				
	0- 8	39	10	11.6	42.14		
Patients	8-17	17	9	5.7	16.55	1.57*	NS
	>17	15	16	2.9	7.64	6.64**	0.036

* $X(1)^2$ of heterogeneity between RR (0-8) and RR (8-17)

** $X(2)^2$ of heterogeneity between RR (0-8), RR (8-17) and RR (>17)

°P value of the X^2 for heterogeneity

The frequencies of HLA-DR3 and -DR4 were the highest in the youngest age category and showed a gradual decrease in frequency in the middle and oldest age category. The differences among the relative risks for HLA-DR3 were not significant. However, inclusion of the oldest age category resulted in significant heterogeneity of the relative risks for HLA-DR4 ($p = 0.036$). Because of the latter heterogeneity, we restricted the further analysis in this report, to the patients with onset of IDD before the age of 17.

Population analysis

75 Unrelated patients with onset before 17 years were analysed for HLA, and compared to the results of normal controls. The results are shown in table 2.

Table 2. HLA antigen distribution in IDD patients (n = 75) and normal controls.

Phenotype HLA!	Patients (n = 75) pos.(%)	Normal controls (n = 1018) pos. (%)	Rel. Risk	X^2	P value	Etiologic fraction
B7	9(12)	282(27.7)	0.4	8.142	0.005	
B8	33(33)	133(24.0)	1.4	14.426	0.000*	0.118
B12	11(15)	260(25.6)	0.5	3.950	0.044	
B15	23(31)	173(17.0)	2.2	8.934	0.003	0.169
B18	12(16)	73 (7.2)	2.5	8.059	0.005	0.095
Bw22	0 (0)	48 (4.7)	0.1	3.952	0.044	
Cw3	36(48)	351(34.7)	1.7	5.460	0.018	0.200
Cw4	7 (9)	215(21.2)	0.4	5.425	0.019	
		(n = 201)				
DR1	10(13)	40(19.9)	0.6	1.466	0.224	
DR2	2 (3)	56(27.9)	0.1	15.785	0.000*	
DR3	46(61)	58(28.8)	3.9	23.297	0.000*	0.457

Table 2 continued. HLA antigen distribution in IDD patients (n = 75) and normal controls.

Phenotype HLA!	Patients (n = 75) pos.(%)	Normal controls (n = 1018) pos. (%)	Rel. Risk	X^2	P value	Etiologic fraction
DR4	56(75)	49(24.4)	8.9	50.933	0.000*	0.665
DR5	3 (4)	35(17.4)	0.2	7.417	0.007	
DRw6	11(15)	59(29.4)	0.4	6.827	0.009	
DR7	4 (5)	50(24.9)	0.2	11.575	0.001*	
DRw8	2 (3)	5 (2.5)	1.2	0.073	0.777	
DRw9	2 (3)	5 (2.5)	1.2	0.069	0.782	
DRw10	0 (0)	10 (5.0)	0.1	3.661	0.053	

! HLA-A, -B, and -C antigens included if significant before correction for number of comparisons

* Significant after correction for 40 comparisons

The frequencies of HLA-DR3 and -DR4 were significantly increased in patients with a relative risk of 3.9 and 8.9, respectively. The corresponding values of the etiologic fraction confirmed the strong positive association with HLA-DR3 and especially with HLA-DR4, and indicated a weaker association with B locus antigens, -B8, -B15 and -B18. HLA-DR2, -DR5, -DR7 and HLA-B7 were decreased among the patients. Taking into account the associations with DR3 and DR4, only DR2 appeared to be decreased significantly (data not shown).

Table 3 shows the analysis of H-W equilibrium among patients and controls. It must be noted that the patients sample is not a true population in the genetical sense, and that within the present patient group heterogeneity might still exist, as some patients come from simplex and others from multiplex families. That this ascertainment difference is not critical as far as the HLA-DR

frequencies are concerned, may be seen from the separate figures shown in table 3.

Table 3. Analysis of Hardy-Weinberg equilibrium in IDD patients (n = 75) and controls (n = 201)

	Patients				Controls			
	OBS.		EXP.	$\frac{(O-E)^2}{E}$	OBS.	EXP.	$\frac{(O-E)^2}{E}$	
	Mult.	Simpl.	Total					
DR3/x	(0+	8)	8	12.48	1.61	48	45.06	0.19
DR3/3	(3+	3)	6	9.01	1.01	5	4.98	0.00
DR3/4	(9+	23)	32	21.49	5.41	5	8.25	1.28
DR4/4	(2+	4)	6	12.81	3.61	3	3.41	0.05
DR4/x	(5+	13)	18	14.87	0.66	41	37.33	0.36
DRx/x	(3+	2)	5	4.32	010	99	101.96	0.09
	(22+	53)	75	74.98	11.95	201	200.99	1.97
				$X(3)^2 = 11.95$			$X(3)^2 = 1.97$	
				$p = 0.0075$			$p = NS$	

Gene frequency patients	Gene frequency controls
DR3:52/150 = 0.3467	DR3 = 0.1574
DR4:62/150 = 0.4133	DR4 = 0.1304
DRx:36/150 = 0.2400	DRx = 0.7122

The gene frequencies of HLA-DR3 and -DR4 did not differ between simplex and multiplex propositi (maximal difference 2 percent), and also the frequency of HLA-DR3/4 propositi is remarkably similar (43 and 41 percent, respectively) for these two categories. Therefore, we pooled the propositi for analysis of H-W equilibri-

um. Whereas the control population did not deviate significantly from H-W equilibrium, the distribution of the allelic combinations in patients was significantly different from the expectation of H-W equilibrium (X^2 = 11.95, p = 0.008). The homozygous DR3/3 and DR4/4 combinations were relatively decreased in frequency. The heterozygote DR3/4 combination was increased in frequency and contributed with 5.41 most to the chi-square value. It must be noted that these calculations are conservative, because in the homozygous DR3/3 and DR4/4 subgroups, the phenotypes with a possible blank allele were included.

Table 4 presents a comparison of HLA-DR phenotypes in patients and controls. A significant increase was found in relative risk for DR3/4 heterozygotes (RR = 26.7) and for DR3 and DR4 homozygotes (RR = 3.3 and RR = 5.3, respectively). The highest odd ratio (OR), as defined by Svejgaard and Ryder (31), among the possible allelic combinations was observed for heterozygote DR3/4.

Table 4. HLA-DR allelic combinations in IDD (n = 75) patients and controls (n = 201)

Phenotype	Patients (75) pos. (%)	Controls (201) pos. (%)	Rel.Risk	X^2	Etiologic fraction	Odd's ≠ ratio
DR3/x*	8 (11)	48 (23.8)	0.4	5.54		3.3
DR3/3	6 (8)	5 (2.5)	3.3	4.42	0.057	23.8
DR3/4	32 (43)	5 (2.5)	26.7	47.99	0.412	126.7
DR4/4	6 (8)	3 (1.5)	5.3	6.75	0.066	39.6
DR4/x	18 (24)	41 (20.4)	1.2	0.48	0.045	8.7
DRx/x	5 (7)	99 (49.3)	0.08	31.71		1.
	75	201				

x* denotes non-DR3, non-DR4

≠ Odd's ratio, calculated according to Svejgaard (31), using DRx/x as reference group

In comparing the different OR's, the OR (DR3/4) vs OR (DR4/4) can be calculated as (32 x 3)/(5 x 6) = 3.2 (exact P = 0.17). Similarly, the OR (DR3/4) vs OR (DR3/3) equaled 5.3 (exact P = 0.036). In addition, the OR (DR3/3) vs (DR3/x) was 7.2 and OR (DR4/4) vs (DR4/x) was 4.6. The latter two values differed significantly from unity with P exact values of 0.01 and 0.04, respectively.

A strong association with DR3 and DR4 and a lower, but significant association with B8, B18 and B15 had led us to consider the question whether the B locus association was part of a real haplotype association, or was rather an association secondary to HLA-DR, due to linkage disequilibrium. To investigate the presence of a haplotype association, we employed the method described by Porta and McHugh (16).

Table 5. Test of HLA-B8-DR3 and HLA-B15-DR4 haplotype association with IDD

Haplotype	Genetic model	Estimate of Delta ≠ haplotype	S.E.°	Z*	P value
B8-DR3	RAD	0.1485	0.0587	2.5310	0.02
	AR	0.0775	0.0336	2.3065	0.02
B15-DR4	RAD	0.1782	0.0513	3.4756	0.01
	AR	0.1054	0.0205	3.7016	0.01

RAD = Rare autosomal dominant

AR = Autosomal recessive

≠ = Delta refers to value of third order linkage disequilibrium

°SE = Standard error

*Z = Denotes the value on the abscis of a normal distribution

We selected two antigen combinations with a positive association in IDD and known to occur in linkage disequilibrium, namely HLA-B8, -DR3 and HLA-B15, -DR4. Both HLA-B8, -DR3 and HLA-B15, -DR4 haplotypes revealed a significant haplotype association in IDD patients, tested in the RAD and the AR model (table 5).

Multiplex families

Twenty-two multiplex families were genotyped for HLA-A, -B, -C, -DR and most families for BF, C2, C4, GLO and GM. 21 Informative families were investigated for segregation analysis of HLA haplotypes.

Table 6 presents the HLA segregation data and it compares the number of HLA haplotypes of the index patient shared in affected and normal siblings. Assuming random segregation, the expected Mendelian ratio is 1 : 2 : 1 for sibs sharing both one or no haplotype with the propositus. A departure of the observed from the expected ratio can be analysed with a chi-square test with two degrees of freedom.

The families were divided in three groups: group A: families with two affected siblings; group B: families with more than two affected siblings; group C: families with two affected siblings and one affected parent.

Affected siblings in group A shared both haplotypes with the propositus significantly more often than expected (X^2 = 22). In group B the relative proportion of sharing one haplotype was increased (X^2 = 4.7) and in group C the observed ratio was not significantly different from the expected (X^2 = 0.33). The normal sibs in group A showed also a significant departure in the other direction from the 1 : 2 : 1 ratio in sharing haplotypes with the propositus (X^2 = 6.8; P = 0.034).

Another observation relates to the occurrence of cross-overs between HLA-DR and the GLO locus, which is located centromeric of HLA-DR at 4 percent of recombination in males (34). Cross-overs in one of an affected sibpair suggest that the gene segment predisposing for IDD is located to the telomeric, HLA-DR side of the

chromosome (32). We observed in our families from a total of 91 informative meioses, six HLA-DR/GLO cross-overs. Two occurred in normal siblings and three in HLA-identical affected sibpairs. One cross-over in a haplotype-identical sibpair was of undetermined parental origin.

Table 6. HLA haplotype sharing with propositus in 21 multiplex families with IDD

Families with IDD in	No of haplotypes shared with propositus							
	affected sibs				normal sibs			
	total	2	1	0	total	2	1	0
A. Two sibs (n=2)	(12)	10	2	0	(30)	2	16	12
B. More than two sibs (n=3)	(7)	4	3	0 +	(9)	1	6	2 +
Subtotal	(19)	14	5	0	(39)	3	22	14
C. Two sibs, one parent (n=6)	(6)	2	3	1 +	(9)	2	6	1 +
TOTAL	(25)	16	8	1	(48)	5	28	15

	affected sibs		normal sibs	
	X^{2}°	P	X^{2}°	P
A	22	0.01	6.8	0.03
B	4.7	0.09	0.9	NS
C	0.33	NS	1.22	NS
A + B	24.89	0.01	6.85	0.03
A + B + C	21.24	0.01	5.5	0.06

°X^{2} = chi-square with two degrees of freedom

The fact that three affected sibpairs were HLA-identical, but different for GLO, confirmed that the "hot" segment for IDD was located towards the HLA-DR side of the chromosome.

Another way of characterizing the HLA-haplotypes associated with IDD is shown in table 7. The unrelated haplotypes encountered in patients ("diabetic" haplotypes) are clustered according to their HLA specificities. In the meantime the same approach has been reported in families from France (35). Three haplotypes were confirmed to occur frequently among the IDD patients.

a) A1, B8, BFS, C2.1, C4AQ0, C4B1, DR3, GLO2
b) Aw30, Cw5, B18, BFF1, C2.1, C4A3, C4BQ0, DR3, GLO2
c) A2, Cw3, B15, BFS, C2.1, C4A3, C4B3, DR4, GLO1

In addition to this observation, the haplotype clusters showed that "historical" cross-overs at the GLO side and the HLA-A side of the haplotypes occurred. Apparently these cross-overs did not change the diabetogenic properties of the haplotypes and inspection of the clusters seems to confine the diabetogenic segment of the B-DR segment.

The gene frequencies of HLA-DR3 and -DR4 (counted from table 7) were lower in the "warm" haplotypes than those in the "hot" haplotypes, but these differences were not significant.

Table 7. Diabetic ("hot" and "warm") haplotypes, in 22 multiplex families, with IDD in two or more IDD patients

FAM	HAPL	H/W	HLAA	HLAC	HLAB	BF	C2	C4A	C4B	DR	LB	LBE	GLOI
011	a	W	33		14	S	1	2	2	1		12	2
003	d	H	24		39	NA	1	.	.	1		12	.
025	a	H	2		39	F	1	3	Q0	1		12	2
012	b	W	24	4	35	F	1	.	.	1		12	2
013	c	H	2		7	S	1	(3)	(1)	2		12	2
013	a	W	2		44(12)	S	1	3	1	2		12	2
010	c	W	3	4	35	S	1	3	1	2		12	1

Table 7 continued.

FAM	HAPL	H/W	HLAA	HLAC	HLAB	BF	C2	C4A	C4B	DR	LB	LBE	GLOI
005	a	H	1	3	8	NA	1	.	.	3		17	2
019	b	H	1		8	.	.	.	.	3		17	2
021	c	H	1		8	.	.	.	.	3		17	2
022	a	W	1		8	.	.	.	.	3		17	.
022	c	H	1		8	.	.	.	.	3		17	.
009	c	H	1		8	S	1	Q0	1	3		17	1
002	d	W	1	3	8	S	1	Q0	1	3		17	2
027	c	H	1		8	(S)	1	Q0	1	3		17	2
025	c	H	1		8	S	1	Q0	1	3		17	2
026	c	H	1		8	S	1	Q0	1	3		17	2
024	a	H	2		8	S	1	Q0	1	3		17	2
012	a	W	2		39(16)	S	1	.	.	3			2
004	a	H	3	5	18	.	.	.	.	3			.
009	a	H	30	5	18	F1	1	3	Q0	3			2
017	d	H	30	5	18	F1	1	3	Q0	3			2
002	c	W	2	4	35	S	1	3	1	3			2
015	a	W	2	3	60	S	1	3	1	3			2
019	c	H	31	3	60	.	.	.	.	3			2
015	b	W	31		51	F	1	3	Q0	4	4	13	1
018	b	W	3		7	.	.	.	.	4	4	13	1
013	b	W	24		7	S	1	3	Q0	4	4	13	2
026	a	W	24		7	S	1	3	1	4	4	13	1
026	b	W	3		14	S	1	Q0	1	4	4	13	
004	c	H	2	3	62(15)	.	.	.	.	4	4	13	.
003	b	H	2	3	62(15)	NA	1	.	.	4	4	13	.
001	b	H	2	3	62(15)	S	1	3	3	4	4	13	1
011	c	H	2	3	62(15)	S	1	(3)	(1)	4	4	13	1
015	c	H	2	3	62(15)	S	1	3	3	4	4	13	1
001	d	H	3	3	62(15)	S	1	3	3	4	4	13	1
024	d	H	2	3	62(15)	S	1	3	Q0	4	4	13	2
012	c	H	11		39(16)	S	1	.	.	4		13	2
027	a	H	2	6	57(17)	S	NA	6	1	4	4	13	1

Table 7 continued.

FAM	HAPL	H/W	HLAA	HLAC	HLAB	BF	C2	C4A	C4B	DR	LB	LBE	GLOI
017	a	H	25		18	S	1	3	1	4	4	13	1
006	b	H	2	2	27	.	.	.	.	4	4	13	1.2
022	b	H	2	2	27	.	.	.	.	4	4	13	.
021	a	H	3	2	27	.	.	.	.	4	4	13	1
002	b	H	3	6	37	F	1	3	1	4	4	13	1
005	c	H	24	6	37	NA	NA	.	.	4	4	13	2
023	d	H	31	3	60(40)	S	1	3	1	4	4	13	1
023	a	H	2		44(12)	S	1	3	1	5	5	13	1
001	a	W	24		18	S	1	3	1	5	5	13	2
018	c	W	2	3	62(15)	.	.	.	.	6		12	1
018	a	W	33	3	58(17)	.	.	.	.	6		12	1
011	b	H	24	3	55(22)	S	2	4	5	6		12	2
010	a	H	11	4	35	S	2	3	1	6		12	1
024	c	W	2	3	60(40)	S	1	Q0	2	6		12	2
006	c	H	2	3	60(40)	.	.	.	.	6		12	1.2
018	d	W	2	6	50(5)	.	.	.	.	7	7	17	1
010	d	H	3		13	S	1	3	1	7	7	17	1

Legend to table 7

HAPL = haplotype

H/W = "hot" and "warm"

LB = subtypes of HLA-DR. LB4 and LB10 are defined as a split of DR4, LB5 and LB58 of DR5 and LB7 and LB11 of DR7

LBE = defined by serum MO which recognizes MB2 + DR3

See for definition of LB and LBE reference 44.

NA = no activity

. = not tested

Q0 = Quantity zero = null allele

Table 8 shows an analysis of non-random segregation for GM to affected siblings. In none of the three families we found evidence for non-random segregation of parental GM haplotypes. Thus, these data do not provide evidence for a predisposing gene for IDD related to genes coding for immunoglobulin heavy chains.

Table 8. Segregation of GM haplotypes to affected children in 21 multiplex families with IDD

Segregation of parental haplotypes in families with	No of families	No of informative parents	SD	Sd	Sd^2	X^2	P
A. Healthy parent, two affected sibs:	12	7	8	7	7	0.036	NS
B. Healthy parent, three affected sibs:	3	4	3	5.5	3.25	1.231	NS
C. Affected parent, two affected sibs:	6	3	2	3	3	0.083	NS
				A + B =		0.098	NS
			A + B + C =			0.3	NS

NS = Not significant

Discussion

Our study describes the role of immunogenetic factors in insulin-dependent diabetes as detected in unrelated patients and multiplex families with IDD in The Netherlands.

We will consider the following aspects in more detail.

1. Genetic heterogeneity related to age of onset of IDD.
2. Associations in unrelated patients.
3. Genetics in multiplex families with regard to HLA-segregation, occurrence of cross-overs and haplotypes.
4. Segregation of GM.

Age of onset.

1. We have investigated the frequencies of HLA-DR3 and HLA-DR4 in IDD patients with regard to age of onset. Comparing three age groups, we found that the frequencies of both HLA-DR3 and -DR4 decreased with older age of onset and that the relative risk of HLA-DR4 was significantly heterogenous. Whereas in patients with onset at adult age, IDD may be diluted with NIDD, we favour another explanation for the observed heterogeneity.
   Because IDD is a disease caused by a mixture of genetic and environmental factors, one might expect that at younger age, where the exposure time to environmental factors is shorter, the genetic factors are more prominent.
   Evidence for this hypothesis was recently reported in a study, which showed an increased recurrence of IDD in sibs of propositi with onset of IDD before the age of ten as compared with that in sibs of probands with onset after the age of ten (36). To make a genetic analysis more meaningful, we excluded for further study the patients with onset of the disease after the age of 17. Although the cut-off point of 17 may be arbitrarily, it coincided with excluding patients from the adult clinic.
2. The patient and control sample were investigated for the presence of H-W equilibrium and the data of the patients were compared with those expected under various genetic models. As stated before, this analysis requires the sample to be chosen randomly from a large random mating population. A priori this assumption may not be fulfilled.
   However, we considered our patient and control population to be fairly homogeneous with regard to ethnic background, because only individuals of Dutch Caucasoid extraction were in-

cluded. Moreover, we reduced heterogeneity within the patients by confining the analysis to patients with onset of the disease before the age of 17. Although the ascertainment was not the same for all the patients, it appeared that the observed gene frequencies and allelic combinations of HLA-DR were very similar in the population and multiplex family sample.

Under the assumption of a single recessive susceptibility gene in linkage disequilibrium with HLA-DR3 and -DR4, the expected proportions of allelic combinations are in H-W equilibrium (37). However, the observed proportions deviated significantly from expected, providing evidence against a single recessive model.

Moreover, the excess of observed over expected HLA-DR3/4 heterozygotes is incompatible with a single dominant or intermediate model (37). In a different approach we compared the odd's ratio's (OR) of the different allelic combinations, employing the method of Svejgaard and Ryder (31). They showed that the OR of the heterozygote DR3/4 would fall between the two homozygote OR's, if the disease were due to a single susceptibility gene acting as recessive, dominant or intermediate gene.

The observed excess of the OR (DR3/4) over OR (DR3/3), provided in another way evidence against a simple recessive, intermediate or dominant model.

Information in determining the mode of inheritance can be also derived from the haplotype segregation in families.

3. Without considering the allelic combination of HLA-haplotypes, the multiplex families demonstrate an excess of sharing both HLA-haplotypes among pairs of affected sibs. The observation that the families with an affected parent less often demonstrate HLA identity among affected sibs than families with two normal parents, can be explained by an increased conditional probability of observing susceptible genotypes in the children of affected parents, as compared to genotypes segregating from normal parents (32). Thus, also the high frequency of sharing both haplotypes in affected sibpairs indicates that a dominant model of inheritance can be ruled out. Moreover, the combined

data of the allelic combinations in unrelated patients and of the haplotype segregation in multiplex families, do not fit a simple recessive or intermediate mode of inheritance. Therefore, more complex genetic models seem to be required to describe the relationship between the HLA system and IDD adequately.

We considered the possibility that HLA-haplotypes rather than a particular antigen were associated with IDD. Based on population data we found evidence that HLA-B8, -DR3 and HLA-B15, -DR4 haplotypes manifested a third order linkage disequilibrium under a RAD and AR model. This observation seems compatible with a susceptibility gene between HLA-B and -DR or with a set of genes with an additive or interactive effect. Although the conclusion of a haplotype association should be taken with some caution, because the patient population is not a true population in genetical sense and the assumptions of the mode of inheritance are only partially valid, the difference of linkage disequilibrium of these haplotypes in patients as compared to normal controls, seems worthwhile in considering the genetics of IDD.

The occurrence of cross-overs between HLA-DR and GLO in one of an affected sibpair enabled us to localize the diabetic segment towards the HLA-DR segment of the chromosome. We cannot, however, determine at present what the distance is of the "hot" diabetic segment to GLO. Markers between GLO and HLA-DR would allow to answer this question, and the newly discovered SB locus (38) may turn out to be a very good candidate for resolving this question (39).

Another way of further characterizing the chromosomal segment associated, is the analysis of haplotypes occurring in affected sibs as shown in table 7.

Sorting of diabetic haplotypes reveals clusters of chromosomal segments selected for being diabetogenic. Inspection of these clusters indicates that "historical" cross-overs have occurred both on the GLO and the HLA-A side of the haplotypes, and that the identity of the segments concentrates around the HLA-B-DR region. Three clusters have been delineated, that previously

were reported to occur in IDD patients (35). Considering that these haplotypes are unrelated, it is striking to see that in these three clusters the identity even seems to extend from HLA-A to GLO. Because, at present we have no appropriate data on control genotypes, the significance of the latter observation remains to be determined.

However, our population data suggest that indeed an increased linkage disequilibrium occurred in patients as compared to normal controls, with regard to the haplotype HLA-B8-DR3 and HLA-B15-DR4.

4. In a search for genetic factors in IDD, not related to HLA, we investigated GM as a marker system, because GM has been implicated in other HLA-associated diseases such as chronic active hepatitis and myasthenia gravis (19,20). In the present families we found no evidence for non-random segregation of GM haplotypes.

   This observation may not be generalized to all families with IDD, because a recent study demonstrated that non-HLA factors may only be prominent in families with a propositus negative for HLA-DR3 and DR4 (40).

Taken together, the results of this study suggest that the HLA system is associated with a major susceptibility effect for IDD. Moreover HLA-DR4, or a susceptibility gene in linkage disequilibrium with it, is associated with age of onset. In addition, the data provide strong evidence against a single recessive, intermediate or dominant mode of inheritance of the susceptibility genes. Therefore more complex models are required to account for the data (40).

With regard to more complex models, it is relevant to consider some molecular aspects of the MHC system. The HLA-DR or class II molecules consist of an alpha and beta chain that are encoded by two closely linked loci (41). In the MHC of the mouse it has been shown that these class II molecules are the products of the immune response genes (42). These alpha and beta loci act in complementation and hybrid alpha-beta molecules of both cis- and trans-complementation can be formed.

In addition to the creation of "new" hybrid molecules as the re-

sult of transcomplementation, it has been shown that preference for cis- or transcomplementation is dependent on the allelic combination of haplotypes (43). This preference is reflected in an immune response gene effect (43).
By analogy, one could suggest that in humans both HLA-DR3 and -DR4 are associated with susceptibility for IDD, and that in a heterozygote DR3/4 a preferential transcomplementation leads to an even increased susceptibility for IDD. However, because this possibility does not easily explain the observed B-DR haplotype association, one should also consider the possibility, that a gene interaction between alleles coding for class I and class II (and perhaps even class III) HLA molecules might be involved in the susceptibility for IDD.
Thus recognition of fine specificities of the HLA-region has also implications for the study of the molecular mechanisms underlying the susceptibility of IDD.

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Paper 2

Neonatal onset permanent diabetes mellitus and incomplete fetal alcohol syndrome: cause or coincidence?

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Summary

Neonatal onset permanent diabetes mellitus is extremely rare and pathogenetic mechanisms are suspected to differ from those described in childhood diabetes developing at later ages. The present child had other HLA-types than mostly seen in childhood diabetes, lacked islet-cell auto-immunity but did have features of the fetal alcohol syndrome. Other (genetic) syndromes associated with diabetes at very young ages were excluded.

Introduction

Since the fetal alcohol syndrome was rediscovered in 1973 (1) a variety of associated anomalies have been reported, including extrahepatic biliary atresia, and renal anomalies (2). We report ketotic diabetes mellitus starting the seventh day of life in a child with features of the fetal alcohol syndrome.

Case report

A 2200 g boy was born in another hospital to a 29 year old mother after an unknown duration of gestation. Although length and head-circumference were not recorded at birth, a "small-for-date appearance" was noted. The pregnancy was complicated by the daily ingestion of unknown quantities of beer and wine, and by the ingestion of half a litre or more of dutch gin per day intermittendly in the third month of pregnancy, during a summer vacation. Previously two healthy children had been born. The mother thought she had taken less alcohol during those pregnancies. Two years before the mother had an oral glucose tolerance test with normal result. This was only done because her mother had developed glucose intolerance at age 60 and the mother herself had complaints of thirst at that time. In the last trimester of this and previous pregnancies no glucose was found in the urine. Except for ampillicin for a urinary tract infection no medication was taken by the mother, as evidenced from her pharmacy's record. She smoked 20-40 cigarettes daily. Lues was excluded. The placenta weighed 400g. Microscopy revealed the partial presence of a single layer of trophoblast-cells, but no other abnormalities.

At birth fetal alcohol syndrome was not suspected. Maximal bilirubin was 65 μMol/l at the fourth day of life. On the seventh day of life mild glycosuria was detected, without ketonuria. This gradually increased up to 20 g per 24 hrs and the child was referred to our hospital at the age of 13 weeks.

At that time weight was 3400 g (10th percentile), length 53 cm ($<$-2 SD), headcircumference 34.2 cm ($<$-2 SD). On physical examination features of the fetal alcohol syndrome were found (1). These are tabulated in table 1.

Table 1

SYMPTOMS IN INDEX CASE COMPARED TO THE FETAL ALCOHOL SYNDROME (FAS)

SYMPTOM:	INDEX CASE	% FAS-PATIENTS SHOWING SYMPTOMS*
GROWTH:		
prenatal length	?	80
postnatal length $\leqslant$ p10	+**	80
adipose tissue $\leqslant$ p10	+	50
CNS-DYSFUNCTION:		
mental deficiency, mild-moderate	+	80
microcephaly	+	80
poor coordination/ hypotonia	+	50
irritability	+	80
hyperactivity	+	50
FACIAL/HEAD:		
short palpebral fissures	+	80
short nose	+	50
hypoplastic philtrum	-	80
maxillar hypoplasia	+	50
thin upper vermillion	-	80
retrognathia	+/-	50
ptosis/strabismus	+	26-50
eye abnormality	-	26-50
ear malposition	+	26-50
prominent palatal ridges	-	26-50
small teeth	+	1-25
CARDIAC:		
defects	-	26-50

Table 1 continued.

URINARY TRACT:		
hypospadia's	-	1-25
small kidneys, hydronephrosis	-	1-25
CUTANEOUS:		
hemangioma's	-	26-50
hirsutism	+	1-25

* data from reference
** + is present; - is absent

Initial laboratory results included: an oral glucose tolerance test (1.75 g/kg) with glucose rising from 8 to 25.4 mMol/l, all insulin values below 1 mU/l, glucagon 36-98 ng/l (normal up to 80 ng/l), growth hormone elevated ranging from 10 to over 40 mU/l. Pre-treatment random blood-glucoses varied from 9-38 mMol, urinary ketones were only present in minor amounts. A 24 hrs urine analysis for amino-acids and oligosaccharides were normal, except for glucose. The diurnal rhythm for cortisol was normal. Chromosome-analysis using trypsin-Giemsa banding was normal male (46, XY). Epiphyseal dysplasia, cystic fibrosis, exocrine pancreas dysfunction, and coeliac disease were excluded. Thyroid function was low normal, TSH never elevated. Tests for liver and kidney function, blood gases, electrolytes and hematology were unremarkable.

Insulin substitution was initiated at the age of 15 weeks in single or multiple dosages not exceeding 0.7 U/kg, using mixtures of short- and intermediate-acting insulin. Despite the careful administration of these little amounts of insulin, blood glucoses bounced from 1.8 up to 25 mMol/l in an erratic fashion. Glycosy-

lated hemoglobins varied from 10-14% (6-8% in normal children) and urinary ketones (2+ or more) were found twice weekly on the average. Frank hypoglycemia occurred two times, the child quickly recovering on oral glucose administration. Keto-acidosis ($pH < 7.2$) was first seen at the age of 6 months during an upper respiratory tract infection. Respiratory tract infections recurred, requiring adenotonsillectomy, ear-tubes for chronic otitis and antibiotics for Haemophilus and Proteus bacteria. Keto-acidosis requiring admission occurred two times thereafter. Recovery with intravenous insulin and electrolyte solutions was normal. Diaper-dermatitis was present, requiring almost chronic local treatment mostly for Candida. Staphylococcus folliculitis was seen four times. Immunoglobulins, including secretory IgA and complement-factors were normal for age. Islet-cell cytoplasmatic antibodies, thyroid antibodies, adrenal antibodies and gastric antibodies were first tested at 8 months, subsequently with yearly intervals. No positive results were obtained, neither in the family. The patients' HLA-D-genotype was HLA-DR1 and HLA-DR5. Hyperreactivity and irritability were almost constantly present. Fine-motor seizures and athetotic movements lasting 5-10 minutes became apparent during the second year of life. Electroencephalography revealed aspecific epileptic activity, for which phenobarbital and phenytoin were prescribed. At present the child is 4 years and 10 months old, has severe growth and mental retardation, and stays in an institution for handicapped children.

Comment

Diabetes mellitus has not been recognized as a symptom of the fetal alcohol syndrome; however, the present case suggests the possibility of such an association.

Permanent insulin dependent diabetes developing before the age of six months is extremely rare. In a recent nation-wide incidence study over three years time only 3/1271 newly diagnosed children from 0-19 years of age became permanently insulin dependent before the age of six months (3). One was the present patient, the other two had multiple congenital malformations, other than seen

in the fetal alcohol syndrome. The term congenital diabetes has been used to indicate failure of fetal development of islets of Langerhans (hypoplasia or aplasia of islets). Only one well documented case is known to have been delivered alive, and it is believed that congenital diabetes is essentially non-existent (4). As glucose-intolerance developed gradually some days after birth in the present case, it may be presumed the neonatal pancreatic response was sufficient during the first week of life to prevent diabetic symptoms. Insulin dependency was documented by hyperglycemia, ketonuria, absolute insulin deficiency, relative hyperglucagonemia and elevated growth hormone levels. These endocrine findings are compatible with selective insulin deficiency. No autoantibodies to islet-cells were found within one year of the clinical onset of insulin deficiency in this patient and his HLA-DR-type was neither HLA-DR3 nor HLA-DR4. These immunologic findings speak against the autoimmune involvement of the islets seen in childhood diabetes: 80% of the newly diagnosed children at older ages have such antibodies at diagnosis and 60% after one year of treatment (5).

Almost all children with an age of onset below 8 years studied in the Netherlands had HLA-DR3 and/or HLA-DR4 haplotypes as well as 90% or more of children studied elsewhere with older ages of onset (6,7). The present patient's HLA-genotype comprised neither of these haplotypes. Other etiologic factors may be supposed in this highly unusual presentation of childhood diabetes, but the known genetic syndromes in which diabetes mellitus concurs were excluded.

The amounts of alcohol ingested during gestation were probably not as high as those reported in cases of children with the fetal alcohol syndrome in which cases permanent alcohol consumption throughout gestation is believed to occur (2). It has been suggested that 90 ml of absolute alcohol per day (equivalent to some six hard drinks) constitutes a major risk to the fetus (7). The present history contained an intermittend alcohol consumption, far exceeding this quantity during the third month of pregnancy by history and there appears enough descriptive evidence (table 1) to at least suppose fetal alcohol effects.

The time sequence as well as the quantities of the gestational alcohol ingestion may be related to the partial expression of the fetal alcohol syndrome of the present case. Hitherto, insulin dependency has never been seen in the full syndrome. This does not preclude interference with the development of the functions of islet-cells during fetal life to a lesser extent than leading to frank insulin dependency: insulin levels in the full fetal alcohol syndrome have not been reported although it is known many of such children brought in at later ages were referred for "being too skinny" and there are no follow-up data with regard to this clinical feature (2).

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Paper 3

Prediction of type-1-diabetes mellitus: a report on three cases.

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Summary

In three children (patients 1, 2 and 3) insulin-dependency was predicted 28, 32 and 4 months before the disease became clinically manifest, respectively, by the finding of islet-cell antibodies at that time.

These retrospective findings confirm the evidence for a long pre-diabetic phase in childhood diabetes, marked by the presence of islet-cell antibodies, as well as the linkage of HLA-antigens to the susceptibility to this disease.
The possibility to detect pre-diabetic states in children before the endogenous insulin secretion decreases to the point of clinical symptoms, supports efforts by basic scientists to develop techniques for immunologic intervention early in the course of this disease.

Introduction

It is well known that diabetes mellitus shows a familial tendency. However, attempts to assess modes of inheritance failed, before it was realized type-1 (insulin-dependent) diabetes mellitus (IDDM) is a distinct disorder from type-2 (non-insulin-dependent) diabetes mellitus (NIDDM) (1).

From the Pediatric Register of the British Diabetic Association it has been estimated that 5.7% of the siblings of diabetic children will develop insulin dependency themselves by the age of 16 years (2). In the Pittsburgh studies (3) the recurrence risks for siblings in families already having a type-1-diabetic child by the age of 20 years was 3.3% to 6.0%, depending on the calculation methods.

In the past decade two factors have been associated with the recurrence risk for siblings of diabetic children: one is HLA identity, on the basis of the fact that the genetic susceptibility to the disease is strongly HLA-linked (4); the other is the presence of islet-cell cytoplasmatic antibodies (ICCA), found in 80% of the newly diagnosed children with IDDM (5).

The question to what extent these two factors might predict future type-1-diabetes in families of affected children has been investigated in the Barts-Windsor Family study (6).

They found that sibs HLA-identical to the already diabetic child, would have a 100 times greater risk of developing the disease themselves, whereas non-HLA-identical sibs would have the same risk as the general population. In that prospective study each of the six siblings that did develop diabetes in the course of their follow-up (up to 4 years at the time of the report) did have ICCA from the moment they entered the study, whereas in a control population 1% positives for the presence of ICCA were found. The study indicated that type-1-diabetes in children has a long prediabetic period and carries implications for future research into preventive measures before the disease becomes clinically mani-

fest.

The present report describes three children retrospectively (patients 1, 2 and 3), in which diabetes mellitus was predicted 28, 32 and 4 months before the disease became clinically manifest, based on the finding of islet-cell antibodies.

Patients

Patient 1

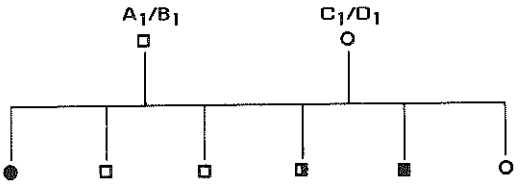
Patient 1 participated in a study on genetic markers in families with two or more children having type-1-diabetes, described in detail elsewhere (7). Blood was also drawn for the determination of ICCA. This study included 22 families, selected from all over the Netherlands on the basis of their willingness to cooperation. It was agreed upon beforehand with each of the 22 families that single blood drawings would be done and that the results of the tests would not be disclosed. The 22 families had 111 children in total, aged 2-20 years (average 11.6 years), 51 of the children were already treated for insulin-dependent diabetes, 60 sibs had no diabetes. Of these 60 sibs patient 1 was the only child, that did have ICCA (titre 1:64), without any sign of clinical diabetes: a random bloodglucose at the moment he participated in the study (fig. 1) was 5.4 mmol/l, his percent glycosylated hemoglobin (HbA_1%) was 6.3% (normal range 6-8%). His disease became clinically manifest 28 months later at age 19 years, with a -retrospective- history of increasing lassitude, thirst and hunger of three months. He was then admitted to another hospital with mild keto-acidosis (blood pH 7.2, urinary ketones 2+, blood glucose 25 mmol/l).

The HLA-segregation of his family is depicted in figure 1, including the titres of (complement-fixing-) islet-cell cytoplasmatic antibodies (CF-ICCA). Islet cell surface antibodies (ICSA) were not measured in this family. The ICCA found in patient 1, indicated by a half-open square, fixed complement in a titre of 8. His already diabetic brother, born in 1964, had a remnant ICCA-titre.

The HLA (-A-B-C-DR) haplotypes are indicated by A_1/B_1 for the father and C_1/D_1 for the mother. Patient 1 is haplo-identical (shared C_1 only) to his older sister and younger brother, already having IDDM, and HLA-identical (sharing both A_1C_1) to his brother born in 1961, who did not develop IDDM to date and did not have ICCA positivity either.

With regard to the family-history of patient 1, no second degree relatives had IDDM, but his father had undergone an operation for hyperthyroid thyroidism 15 years earlier.

Fig. 1 HLA-segregation, islet-cell antibodies and glycosylated hemoglobins found in the family of patient 1 in January 1980 (legend see text).



year of birth	1958	1961	1962	1963	1964	1965
year of IDDM	1960	-	-	1982	1978	-
HLA-antigens	A_1C_1	B_1/C_1	B_1/D_1	B_1/C_1	A_1/C_1	B_1/D_1
ICCA-titre	0	0	0	64	4	0
CF-ICCA titre	0	0	0	8	0	0
HbA_1 %	12.9	6.1	7.4	6.3	15.1	6.8

patient 1

female IDDM

male IDDM

Legend to figure 1.

	HLA			
	A	B	C	DR
A_1 =	2	8	-	3
B_1 =	11	W35	W4	1
C_1 =	2	W15.2	W3	4
D_1 =	2	W40.1	W3	W6

None of the other 59 non-diabetic siblings of this multiplex family study, all being negative for (CF-)ICCA, developed IDDM over a three year timespan, from the moment each child entered the study. The bloodsample of patient 1, obtained 28 months prior to his insulin dependency and another sample obtained one month thereafter, showed no significant difference in virus-titres for mumps, measles, german measles, cytomegalia, adenoviruses, reoviruses, no tests were done for titres against coxsackie-virus.

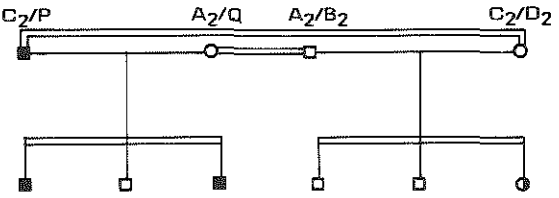
Patient 2

Patient 2 is a girl of 14 years old when IDDM developed clinically. She is the cousin of two IDDM children that took part in the multiplex family study aforementioned. However, neither she herself nor her first degree relatives had diabetes, but it so happened her father's sister was the mother and her mother's brother was the father of her two IDDM cousins (figure 2). Because of this relationship it was decided to draw blood of her family too, the HLA-haplotypes found in these two families are in table 2.

Patient 2 had no (CF-)ICCA in March 1979 (fig. 2) but 32 months later, at the clinical onset of her disease, an ICCA-titre of 8 was found, that fixed complement with a titre of 2. Because of the absence of ICCA in March 1979 this serum was assayed for islet-cell surface antibodies (ICSA) on beta-cells obtained by cell-sorting techniques from rat-islets (8). Islet cell surface antibodies (ICSA) were 3+, in the absence of cytoplasmatic antibodies (ICCA). Two years after treatment the ICCA, present in a

titre of 8 at diagnosis, had increased to 16. Her HLA-identical cousin, born in 1961 had "persisting-ICCA", not usually seen after a duration of IDDM for ten years (5).
As indicated in figure 2 her uncle also had IDDM. He developed the disease at age 29, but no other more distant relatives were insulin-dependent nor had other endocrine autoimmune diseases, been reported. The same virus-titres as in patient 1 were obtained, but the results were unremarkable.

Fig. 2. HLA-genotypes, islet-cell antibodies and glycosylated hemoglobins, found in patient 2 and her relatives in March 1979 (legend, see text).



year of birth	1961	1966	1967	1963	1965	1968	
year of IDDM	1972	-	1969	-	-	1982	
HLA-genotypes	A_2/C_2	C_2/Q	A_2/C_2	A_2/D_2	B_2/C_2	A_2/C_2	
ICCA-titre	16	0	0	0	0	0	(1:8)
CF-ICCA titre	4	0	0	0	0	0	(1:2)
ICSA	-	-	-	-	-	+	
HbA_1 %	13.2	6.0	11.3	5.6	5.7	6.0	

Legend to figure 2.

HLA-haplotypes found in patient 2 and her relatives

		HLA types			
		A	B	C	DR
A_2	=	1	8	W3	3
B_2	=	11	44	W2	3
C_2	=	2	27	W2	4
D_2	=	29	18.4	W3	5
P	=	2	8	W2	3
Q	=	3	7	W2	2

Patient 3

Patient 3 is a boy, aged 3 3/12 years, when insulin was first administered. He is the only son of a type-1-diabetic mother who had developed the disease herself at age 27 years. This patient came to our attention because of glucosuria and hyperglycemia ($\geqslant$13 mmol/l) without ketonuria, seen only during corticosteroid treatment for severe bouts of asthma. For this he received 2 mg/kg prednisone per day as well as 20 mg/kg aminophylline per day. Such bouts lasted 4-7 days, after withdrawal of the corticosteroid treatment he became normoglycemic.

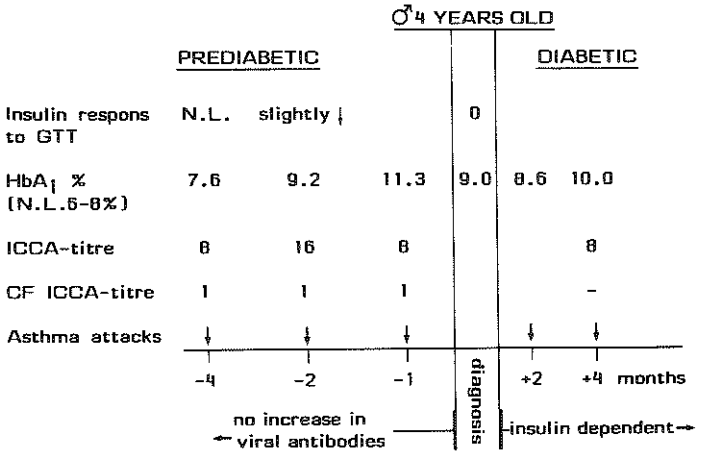
Three days after the first episode an oral glucose tolerance test (OGTT) was done, yielding a peak glucose of 9.7 mmol/l, peak insuline of 13 mU/L and C-peptide increasing from 0.2 nmol/l to $>$ 2 nmol/l. Glucagon and cortisol were 9-14 ng/l, respectievely 0.27-0.41 µmol/l. Five non-diabetic children between 3 and 5 years of age had 6.3-7.8 mmol/l peak glucose, 10-24 mU/l peak insulin and $>$ 2 nmol/l C-peptide on OGTT, also with 1.75 g glucose/kg as the glucose load, with a maximum of 50 g.

Three months later (with the aminophylline treatment continued at 5 mg/kg/day, without corticosteroid treatment in between) the

OGTT was repeated, also three days after corticosteroids for another asthmatic attack were stopped. This time the peak glucose on OGTT, performed as above, was 23.8 mmol/l, peak insulin 1 mU/l, peak C-peptide 0.28 nmol/l. This time normoglycemia did not return: home made glucose measurements varied 4.4 till 22.2 mmol/l despite a diet avoiding oligosaccharides, but ketones were not found in the urine. Three weeks thereafter another asthmatic episode followed, necessitating insulin administration on the basis of hyperglycemia, ketonuria and thirst in the absence of corticosteroid treatment.

The clinical and biochemical data, including the findings on ICCA in this patient are summarized in fig. 3. In addition to the ICCA found when first tested, ICSA could be demonstrated at the same time. Virus tests, made at every time point ICCA were measured (fig. 3), were unremarkable as in patients 1 and 2.

Figure 3 Time-course and biochemical findings in patient 3



As patient 3 is an only child, his HLA-type cannot be compared to that of sibs. His mother had HLA-DR3/DR3, his father HLA-DR2/DR4, patient 3 himself had HLA-DR3/DR4.
Apart from his mother's IDDM since the age of 27, his father had slight asthmatic symptoms as a youth, declining when he became an adult. There were no other family-members with known (auto-immune) endocrine disease.

Methods

The HLA-typing was performed by the standard microcytotoxicity assay, using periferal lymphocytes: 16 HLA-A, 40 HLA-B and 6 HLA-C antigens were determined, using a set of 120 well defined antisera (9). With the two-coloured fluorescence assay 10 HLA-DR antigens were investigated, using 80 different allosera (10). Intra-HLA cross-overs were not observed.

Islet-cell cytoplasmatic antibodies (ICCA) were measured by indirect fluorescence on fresh frozen human pancreas sections (11), complement fixation of ICCA was determined in addition in each sample (5). Islet-cell surface antibodies (ICSA) were estimated on dispersed islet-cells from rodents, as described by Lernmark et al. (12). In patient 2 this assay was performed after separating the pancreatic beta-cells of rodents by cell sorting techniques (8).

Virus-titres were estimated by standard serologic technique, glucose by specific enzymatic reaction (GOD), hormones by radio-immuno-assay, (stable) glycosylated hemoglobins (HbA_1) by a modification of the microcolumn (Isolab^R -Akron (Ohio) USA) chromatography, drawing the blood in excess buffer prior to assay, the normal range is 6-8% HbA_1.

Discussion

Patient 1 illustrates that recurrence of IDDM may occur in haploidentical sibs and that the other healthy sib to which this patient was HLA-identical in turn, did not have islet cell antibodies. Further, patient 1 was the only healthy participant in the

multiplex family study that did have ICCA and did develop IDDM. Thus standardized ICCA (11) may be a good screening agent for prediabetic states of IDDM (12).

Patient 2 had no ICCA 32 months before she came down with IDDM, but she did have ICSA. When her disease became manifest she had low titres of (CF-)ICCA, suggesting that ICSA developed previous to ICCA. This is consistent with the view (14) that cytoplasmatic antibodies (ICCA) would not react with intact pancreatic islets, but only after they have been previously damaged by surface antibodies (ICSA). To the best of our knowledge, this is the first patient in which this supposed sequence of events has actually been documented. The opportunity to study the HLA-segregation in her family, with her parents married to each others brother and sister, demonstrates the association between the susceptibility to IDDM in childhood and HLA-antigens in another way than by concurrence of HLA-antigens in affected siblings. Yet, only 12% of the healthy sibs that are HLA-identical to affected children in the same family, will develop IDDM before the age of 20 (15).

Patient 3 indicates that ICCA may be used in clinical instances where the development of IDDM is suspected. It has been suggested before that corticosteroid-treatment may precipitate the development of IDDM (16), which is in contrast to reports indicating that corticosteroid-treatment, initiated as soon as the diagnosis is made, may have a preservative effect on the residual capacity of pancreatic beta cells to secrete insulin (17).

This contrast exemplifies the necessity to elucidate first pathogenetic mechanisms leading to IDDM, before immunosuppressive therapies are considered in individual patients. Alternatively, the maintenance aminophylline treatment of this patient (5 mg/kg/day) may have stimulated his declining capacity to secrete insulin to some extent: theophylline, by in vitro studies on islet-cells in culture, is a known pancreatic beta-cell secretagogue.

The reports of these children confirm that pre-diabetic states can be detected by the presence of islet-cell antibodies and that the susceptibility to the disease is HLA associated. However, the pathogenetic significance of neither association has been established.

Recently two protein-fractions have been isolated from human islet-cells, reacting with immuno-reactive serum components of diabetic children (18). These protein-fractions may be used to develop more sensitive radio-immuno-assays for the detection of islet- cell antibodies. Also hybridoma techniques, after dissecting the DNA of the HLA-DR4 region with suitable endonucleases, yielded DNA-sequences which may lead to a better understanding of the role of the HLA-genes in the pathogenesis of IDDM in childhood (19).

With these novel approaches the nature of islet-cell antibodies and the genetic mechanisms of the susceptibility may be further elucidated. If so, prospective studies in sibs of diabetic children may be considered exploring possibilities for preventive immunologic measures, on the basis of defined abnormalities to be corrected. In particular, the recently described techniques to vaccinate against animal-disease models for encephalitis, rheumatoid arthritis and thyroiditis should be explored with respect to IDDM (20).

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Paper 4

Clinical time-course and characteristics of islet-cell cytoplasmatic antibodies in childhood diabetes.

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Summary

Circulating islet-cell antibodies (ICA) were present in high frequency (80%) early after diagnosis and decreased in the time course of childhood diabetes mellitus. The complement fixing ability of islet-cell antibodies (CF-ICA) in the course of the disease appeared to depend on the titre of ICA: the coefficient of correlation between ICA and CF-ICA titres was 0.79 and all ICA's with a titre over 16 were complement-fixing. Incubating fresh-frozen human pancreatic sections thrice rather than once with the children's sera, increased the detectability of comple-

ment fixation by a factor 1.4 in all ICA-positive sera. Thus tested, the detection of complement fixation per se did not appear to have a separate pathogenic significance, as the fraction of complement fixing ICA's was almost constant throughout the clinical course. The presence of ICA-IgG subclasses also was dependent on the ICA titre: above a titre of 16 mostly all four subclasses could be detected. Incubating the pancreatic tissue thrice rather than once with ICA-positive sera resulted in enhanced detectability of ICA-IgG_1. Early in the course of childhood diabetes, including two prediabetic children, most of the IgG subclasses could be detected in ICA, but after a duration of one year IgG_1 alone was mainly seen. In two other children, having a family history of insulin-dependency, restriction to the IgG_2 subclass was found.

Introduction

It has been demonstrated that complement-fixing islet-cell antibodies CF-ICA) relate more closely to the clinical onset of childhood diabetes mellitus than non-complement fixing islet-cell antibodies (ICA). Importantly, CF-ICA have been found in siblings of diabetic children, up to 4 years before their disease became manifest (1). There is evidence that CF-ICA encomprise beta-cell cytotoxic antibodies (2), thereby explaining the closer relationship of CF-ICA, rather than ICA, with the actual onset of the disease. Also, CF-ICA tend to disappear more quickly than ICA in the course of the disease (3). However, the ICA-IgG from sera not fixing complement may react with fewer epitopes in the islet-cells, so that the IgG bound would be too far apart to fix sufficient complement components, especially C_3. To test this hypothesis, fresh-frozen human pancreatic sections were incubated once or thrice with the patient's sera, to augment the amount of IgG-antibodies reacting with antigens in the islet-cells. Subsequently, the ability to fix complement as well as the presence of IgG subclasses in the bound ICA was tested after single or triple serum incubation.

Previously, we compared our methods of detecting ICA between two laboratories (4).
We now report our findings on the occurence of complement fixation and IgG subclasses during childhood diabetes, including findings in two children who had CF-ICA 28 and 4 months before the clinical onset of diabetes.

Patients and sera

Sera were obtained from consecutive referrals to the Sophia Children's Hospital of newly diagnosed children (1980-1982) and included follow-up samples (Tables 1, 2 and 4). Figure 1 and Table 3 include samples of recently diagnosed children from other paediatric units (1982). All samples were spun immediately after clotting, frozen at -20°C and assayed within 72 h for ICA. For all other studies, sera were stored up to 3 years before assay. None of the children had thyroid or adrenal disease.
The 25 newly diagnosed children (Tables 1 and 2) had an average age of 9.2 years (range 3-17 years), the male:female ratio was 1:1. On first referral, stable glycosylated haemoglobin levels (HbA_1) averaged 14% (range 10%-17%); 17 children had ketonuria and three had ketoacidosis.
Two patients (1, 2, Table 4) were CF-ICA positive 28 and 4 months respectively before the diagnosis of clinically overt diabetes, necessitating the administration of insulin. The data from these patients are omitted from Tables 1, 2, 3.
Patient 1 (aged 17 years) participated in a study on genetic markers, involving 22 families with two or more children (in total 51) with treated diabetes. These families also had 60 siblings without diabetes. This patient was the only sibling with CF-ICA without any sign of diabetes, a random blood glucose was 5.4 mmol/l, HbA_1 6.3% (normal range 6%-8%), the disease becoming manifest only 28 months later. None of the other healthy siblings developed diabetes over a 3-year time span.
Patient 2 is a boy, aged 3 years 3 months when insulin was first administered. He is the only son of an insulin-dependent diabetic mother, coming to our attention because of hyperglycaemia

(>13.3 mmol/l) during corticosteroid treatment for severe bouts of asthma. Such bouts lasted 4-7 days and after withdrawing corticosteroids he was normoglycaemic. Three days after the first episode, an oral glucose tolerance test (1.75 g glucose/kg) yielded a peak glucose of 9.7 mmol/l, a peak insulin of 13 mU/l and an increased C-peptide > 0.2 nmol/l. Glucagon and cortisol were 9-14 ng/l and 0.27-0.41 µmol/l, respectively. Five non-diabetic children in this age group had 6.3-7.8 mmol/l peak-glucose, 10-24 mU/l peak insulin and >2 nmol/l peak C-peptide during an oral glucose tolerance test. Three months later the oral glucose tolerance test was repeated 3 days after the last corticosteroid dose: by that time peak glucose was 23.8 mmol/l, peak insulin 1 mU/l, peak C-peptide 0.28 nmol/l. This time normoglycaemia did not recur: home-monitored glucose levels varied from 4.4 to 22.2 mmol/l, despite a diet avoiding oligosaccharides. Three weeks later he had another asthmatic episode, necessitating insulin administration since then.
Glucagon, cortisol, insulin and C-peptide were measured by radioimmunoassay, glucose by GOD, glycosylated haemoglobin by microcolumns (Isolab, Akron, Ohio, USA), with the blood drawn in excess saline or buffer prior to assay.

Methods

Assay procedure for ICA

For all studies one pancreas specimen was used (designated as "HP" in the study by Marner et al (4)). The preparation and storage of the pancreas and the method of assessing ICA in terms of titres (defined as the maximal dilution at which fluorescence can be detected) has been described (4). Fluorescence readings were made by two observers, discounting very weak (±) fluorescence. In cases of doubt, involving <2% of all readings, the vote of a third observer was sought. All measurements were carried out as single determination, unless otherwise indicated.
ICA was determined with fluorescein-isothiocyanate (FITC)-labelled rabbit anti-human IgG, IgA and IgM sera specific for the

heavy chain, purchased from the Central Laboratory of the Blood transfusion Service, Amsterdam, the Netherlands (anti-IgG, batch number: KH-16-105-F01, anti-IgA, batch number: KH-14-17-F01, anti-IgM, batch number: KH-15-21-F01). A Leitz Orthoplan microscope (Wetzlar, FRG) with epi-illumination (100 Watt lamp) and dichroic mirror RKP 510 with filter LP 515 (Wetzlar, FRG) were used.
In two out of 46 samples IgG-rheumatoid factors were found (5). Those samples were excluded from the study.

Complement-fixing ICA

Since complement binding to IgG is dependent on the close proximity of IgG molecules, the occurrence of CF-ICA was tested under two different conditions, i.e. pancreas sections were incubated once (complement-fixing-titre) and thrice (complement-fixing-property) with preheated (30 min, 56°C) patient sera (50 µl) for 30 min. The sections were washed with phosphate buffered saline (PBS, pH=7.2) and incubated with human serum as a complement source (fresh serum, blood group AB, stored -80°C, diluted 1:5). After another washing procedure the presence of C_3 was demonstrated by incubation with FITC-conjugated rabbit anti-human C_{3c} (1:80 dilution, Dakopatts A/s, Copenhagen). Fluorescence was evaluated as decribed above.
The conjugate did not react with human IgG, nor could fluorescence be demonstrated under the above conditions, if ICA-positive samples (titres 8-64) were incubated thrice with tissue in the absence of C_3.

ICA-IgG subclass detection

For the determination of IgG subclasses in ICA, specific rabbit anti-human IgG subclass sera were used. The principle of the preparation of these sera has been described (6): they were made specific in agglutination and subsequently in indirect immunofluorescence by adsorption with a panel of relevant isolated IgG-paraproteins and tested against IgG subclass-paraproteins bound to erythrocytes by tannin. When applied to bone marrow smears ob-

tained from multiple myeloma patients, these subclass antisera appeared suitable for use by indirect immunofluorescence. The sensitivity and specificity of these anti-sera (anti-IgG_1, batch number: KH 161-02-A_1; anti-IgG_2, KH 162-19-A_6; anti-IgG_3, KH 163-01-A_1; anti-IgG_4, KH 164-05-A_3, obtained from the Central Laboratory of the Blood Transfusion Service, Amsterdam) was found adequate in immunofluorescence, as well as in an enzyme-linked immunosorbent assay (Elisa) in two other studies (7,8).
To demonstrate the occurrence of a particular IgG-subclass, ICA-positive sera were incubated once or thrice with pancreatic tissue and subsequently tested for the subclasses. Anti-IgG subclass sera were used at 1:20 dilution (excepting anti-IgG_2, 1:40) and were applied in a double layer method in conjunction with FITC-labelled swine anti-rabbit Ig (1:80 dilution, F205, Dakopatts, Copenhagen). The latter did not cross-react with human IgG, as demonstrated by the absence of fluorescence when tested directly on bone marrow cells of 10 multiple myeloma patients or when tested on pancreas sections bound with ICA. Finally, extensive absorption of the conjugate with human IgG did not affect in IgG subclass detection.

Results

Table 1 indicates the prevalence of ICA and CF-ICA during childhood diabetes. Each sample assayed came from a different child. At the onset of disease the prevalence of ICA was 80%, but after a duration of 4 years or more, only 11% of the children had circulating ICA. All positive sera contained ICA of the IgG class. In addition, two sera contained ICA-IgA. ICA-IgM were not found.

If the sera were incubated once with pancreatic tissue, complement fixation was found in 46% (19/41) of the samples (Table 1), but triple incubation of the same sera increased the percentage fixing complement by a factor 1.4 (to 27/41). Of 100 healthy adult blood donors one sample was weakly positive for ICA and none for CF-ICA.

A significant linear correlation could be demonstrated between the fluorescence intensity score observed with undiluted sera and the ICA titre of 32 samples tested ($p < 0.05$). Thirteen samples with a titre $\geqslant 8$ were CF-ICA positive after single incubation. Only two of the 20 samples with an ICA titre $\leqslant 8$ were CF-ICA positive on single incubation, the standard procedure. Triple incubation resulted in four additional CF-ICA positives among these 20 samples with ICA titres $\leqslant 8$.
None of the sera with CF-ICA activity on single incubation lost this capacity by triple incubation.

Table 1. Prevalence of islet-cell cytoplasmatic antibodies (ICA) in the sera of diabetic children in relation to the duration of diabetes.

Months after diagnosis	Number of patients studied	ICA-positive	ICA-fixing complement	
			After one serum incubation	After three serum incubations
0- 3	25	20/25 (80%)	12/20 (60%)	15/20 (75%)
4-12	13	8/13 (62%)	1/8 (13%)	4/8 (50%)
13-24	12	2/12 (17%)	1/2 (50%)	1/2 (50%)
25-48	19	7/19 (37%)	3/7 (43%)	4/7 (57%)
48	37	4/37 (11%)	2/4 (50%)	3/4 (75%)
Total	106	41/106	19/41	27/41
Healthy records	100	1/100 (1%)	0/1 (0%)	0/0 (=%)

Table 2. Follow up of islet-cell antibody (ICA) and complement-fixing islet-cell antibody (CF-ICA) titres 0-12 months after diagnosis.

Patient no.[a]	ICA/CF-ICA[b] titres			
	0 months	0-5 months	5-10 months	8-12 months
1	32/2(0)	32/4(3)	64/16(7)	
2	2/0(0)	2/0(2)		2/0(11)
3	8/0(0)	0/0(2)	0/0 (5)	0/0 (8)
4	64/2(0)	64/4(2)	16/1 (5)	16/1 (9)
5	8/1(0)	8/1(2)	16/4 (7)	
6	32/2(0)	16/2(3)	8/1(10)	8/0(12)
7		16/4(5)	16/8 (8)	8/4(11)
8	16/4(3)	16/2(3)	8/2 (6)	

[a] Eight patients (randomly selected from the 25 (0-3 months) of Table 1); [b] CF-ICA titres obtained after single incubation of the sera follow the corresponding ICA-IgG titre and the exact number of months after diagnosis is shown in parentheses

Of ten samples that lost their complement fixation by dilution 1:3, the complement fixing property reappeared in nine of them after triple incubation. In the sera of 25 healthy adult blood donors no complement fixation could be detected after triple incubation with the pancreatic tissue.

The sequential titres of Table 2 show a gradual decrease of ICA as well as CF-ICA titres during the first year of diabetes treatment. A strong correlation was found between ICA and CF-ICA titres obtained after single incubation (Spearman's rank correlation gave a rho of 0.79, $p < 0.0001$). The CF-ICA titres were mostly much lower than those of ICA-IgG.

Table 3 shows the relationship between ICA titres and IgG subclass detection. The first samples of 44 recently diagnosed diabetic children (0-12 months after diagnosis) were tested for

ICA-IgG subclasses after single or triple incubation of the tissue with the serum.

Firstly, we considered samples that fixed complement after single incubation and in which ICA-IgG subclasses were determined by single incubation. In this category samples with an ICA-IgG titre >16 had most IgG subclasses detectable. Secondly, of the 27 samples with ICA-IgG titres $\leqslant 8$, the number of IgG subclasses detected exceeded two in only three samples, all of which fixed complement on single incubation.

Finally, the 17 samples that either did not fix complement at all or only after triple incubation all had an ICA titre of $\leqslant 16$ and in six of these sera no IgG subclass was detectable, regardless of the incubation procedure.

The above findings raise the question whether the detectability of specific IgG subclasses may be enhanced or suppressed by single or triple serum incubation. IgG subclasses were detected in 37 of the 44 ICA-positive samples of Table 3, after single or triple incubation. Of those 37 sera, 34 contained IgG_1, 17 IgG_2, 18 IgG_3, 10 IgG_4.

ICA-IgG_1 was detected in 9/27 sera with ICA-IgG titre $\leqslant 8$, while triple incubation resulted in the detection of 20/27 ICA-IgG_1. Only in one sample of this category ICA-IgG_1 was lost by triple incubation. The chi square test for paired observations gave $p < 0.05$ for this comparison. Thus, IgG_1 detectability was enhanced by triple incubation in CF-ICA. In CF-ICA negative samples, however, ICA-IgG_1 detectability appeared in 3/10 samples by triple incubation and disappeared in 2/10.

With the other IgG subclasses no significant suppression or enhancement of detectability was found by single or triple incubation.

Seven patients were followed up from before their diagnosis up to 20 months thereafter (Table 4). Despite variability in the detection of IgG subclasses caused by single or triple incubations, consistency of IgG subclasses was found in parts of the clinical course. Changes in subclasses were seen in patients 1, 2 and 5 where a 16-fold decrease in ICA-IgG titre was found.

In all patients, excepting patient 6, IgG_1 was the predominant

ICA-IgG subclass detected in the time course of their disease. This result is in agreement with the quantitative distribution of IgG subclasses found in the sera of 50 normal children, aged 8-12 years, using the same antisera (IgG_1:IgG_2:IgG_3:IgG_4 = 70:20:8:4). Ten randomly selected children with treated diabetes had a similar IgG subclass distribution to that found in the sera of normal children (data not shown).

Patient 6 (encircled in Table 4) with ICA-IgG_2 alone and another patient (encircled in Table 3) having ICA-IgG_2 only on single as well as triple serum incubation, had family histories of insulin-dependency. The maternal grandfather of the first patient was insulin-dependent at age 27 years and his maternal uncle developed diabetes at age 10 years. The other patient had two maternal grand-uncles with insulin-dependency as of ages 36 and 49 years. The sera of these two patients, however, contained normal quantities of IgG subclasses.

Table 3 The distribution of IgG subclasses (1, 2, 3, 4) in relation to ICA-IgG titre and complement fixation in sera of newly diagnosed diabetic children.

Number of incubations	CF-ICA negative		CF-ICA positive on triple incubation only		CF-ICA positive on single incubation	
	One	Three	One	Three	One	Three
ICA-IgG titre	Overall result		Overall result			Overall result
256					4	1+2+4	1+2+4
128							
64					-	1+3	1+3

Table 3 continued.

Number of incubations	CF-ICA negative			CF-ICA positive on triple incubation only		CF-ICA positive on single incubation		
	One	Three		One	Three	One	Three	
ICA-IgG titre			Overall result		Overall result			Overall result
32						(2)	(2)	(2)
						2+3	1+2+3+4	1+2+3+4
						1+2+3+4	1+2+3+4	1+2+3+4
						1+2+3+4	1+2+3+4	1+2+3+4
						1+2+3+4	1+3+4	1+2+3+4
						1+2+3+4	1+3+4	1+2+3+4
16	1	1	1			1+2+3	1	1+2+3
	2	1+2	1+2			1+3	1	1+3
						1+2	1	1+2
						1+2+3+4	1+2+3	1+2+3+4
						1+3	1+2+3	1+2+3
						1+4	1	1+4
						1	-	1
8	-	1	1			-	1	1
						-	-	-
						-	1	1
						-	1+4	1+4
4	-	-	-					

Legend to Table 4

nd = not detectable

[a] Five patients (3-7) were randomly selected from the 25 of Table 1 and children 1, 2 were those in whom ICA was measured before the disease became clinically manifest; [b] IgG subclasses are given as the overall result of their detection by single and triple incubation; [c] titres are those obtained after single incubation of the sera with the tissue. All measurements were done in triplicate and the average titre reported. The reproducibility was always within one titre difference; [d] after triple incubation only

Discussion

The complement fixing nature of ICA may be particularly relevant for the pathogenesis of childhood diabetes as this variety of ICA has been detected in children before the disease becomes clinically manifest (1). This association is confirmed by two more cases in the present study. In clinically overt childhood diabetes, no complement fixation was found if the ICA-IgG titre was 4 or lower, whereas the correlation between ICA-IgG and CF-ICA titres was high, suggesting a limited sensitivity of our system for the detection of CF-ICA titres.

The fixation of complement depends on the proximity of IgG-antibodies bound to the islet cells of the pancreatic sections. Triple incubation resulted in the detection of 1.4 times as many complement fixing ICA as single incubation (Table 1), primarily because samples with ICA-IgG titres of 4 or lower appeared to fix complement by this procedure (Fig. 1). If CF-ICA-positive samples were diluted until the complement fixing detectability disappeared and were subsequently incubated thrice at that dilution, the complement fixing property reappeared. This finding raises the question whether the complement fixing nature of ICA has any significance other than that it is more likely to be seen in samples with higher ICA-IgG titres.

As in other studies, ICA decreased with the duration of the diabetes. However, our fraction of ICA's fixing complement remained

50%-75% (Table 1). Therefore, we do not feel the detection of complement fixation per se has a separate significance.

Dean et al. (9) recently investigated ICA-IgG subclasses and found 50% of the ICA-positive sera showed a restricted response, with 12% reacting only with the IgG_2 subclass. We too found an uneven distribution of IgG subclasses, however some apparent restrictions were clearly dependent on the ICA-IgG titre on which in turn the CF-ICA titre depended. Dean et al. found the restrictions to be independent of CF-ICA titres, although their reported CF-ICA titres were much higher than those in the present study. We used triple incubation of the pancreatic sections to enhance the detectability of complement fixation and if samples demonstrating all four ICA-IgG subclasses were diluted, IgG_1 was the last subclass to disappear. Conversely, triple incubation significantly enhanced the detectability of IgG_1. In addition, the IgG_1 subclass appeared predominant in 13 ICA-positive sera not fixing complement, which suggests again that the IgG_1 subclass was relatively more abundant in ICA, than in whole serum. The absence of detection of any IgG-subclass in seven sera of lower ICA-IgG titre could be due to the limited number of epitopes present in ICA which are specific for the relevant subclasses. The finding that ICA with lower titres did not fix complement may be due to the low density of ICA bound from those sera. The discrepancies indicated may be resolved with the availability of monoclonal IgG subclass-specific antisera and sensitive enzyme-labelled or radiolabelled assays (10), now that two human ICA-reactive islet-cell protein fractions have been separated (11).

In conclusion, indirect immunofluorescence carried out on one single fresh-frozen human pancreas, with a previously established specificity and sensitivity for the detection of ICA (4), yielded variable results for the detection of complement fixation and IgG subclasses, depending on ICA-IgG titres and incubation procedures. The present data suggest the occurrence of a non-restricted IgG subclass response early in the course of childhood diabetes. However, IgG_2 subclass restriction in ICA was seen in two children having a family history of insulin-dependency.

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Paper 5

Autoantibodies to the insulin receptor in juvenile onset insulin-dependent diabetes.

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Summary

Insulin-dependent diabetes mellitus (IDDM) usually begins in childhood or early adulthood, and its aetiology is thought to involve autoimmune damage to the islet-cells that secrete insulin (1). To investigate an additional target of autoimmunity in IDDM we examined sera for antibodies to insulin receptors. Such antibodies were defined by their ability to compete with insulin for binding to insulin receptors and by their capacity to behave like insulin in activating lipogenesis in adipocytes. We now report the occurrence of anti-insulin receptor antibodies of the IgM class in the sera of 10 of 22 IDDM patients obtained before their treatment with exogenous insulin. Furthermore, two of five IDDM patients who were initially negative developed anti-insulin receptor antibodies during treatment with human or pork insulin. These findings suggest that autoimmunity to the insulin receptor may contribute to the pathophysiology of IDDM.

Results and discussion

The population of IDDM patients we examined comprised children who were referred consecutively to the Department of Endocrinology of the Sophia Children's Hospital. Sera were obtained from 22 patients before treatment and from 5 of them also after they began to receive insulin. Control sera were obtained from healthy volunteer blood donors at the Blood Bank of the Leiden University Hospital and from the investigators. We surveyed the sera by assaying 0.5-5 µl for insulin-like activity in stimulating lipogenesis in rat adipocytes measured by incorporation of radiolabelled glucose into lipid (2,3).

Table 1 shows that the insulin-like activity in a representative serum could be identified as anti-insulin receptor antibody of the IgM class by fractionating the serum and testing the various fractions for lipogenic activity. For example, 75% of the lipogenic activity found in the whole serum could be eluted from an anti- µ-column that specifically binds IgM. The effluent which contained the non-IgM material had relatively little lipogenic activity. Filtration through Sephacryl 300 to separate IgM from IgG confirmed that the lipogenic activity was a property of the fraction containing IgM, while the fraction containing IgG was inactive. Dissociation of possible insulin-insulin antibody complexes by acidification and separation of serum fractions on Sephadex G-100 showed that the lipogenic activity remained intact and resided in the insulin-free, high-molecular weight fraction (2). The low-molecular weight fraction did not contain sufficient insulin to activate lipogenesis. The lipogenic activity of the positive sera was not inhibited by adding antibodies to insulin, illustrating by another method that the lipogenic activity was not due to insulin itself. In contrast, goat antibodies to human IgM inhibited about 95% of the lipogenic activity in the positive sera. Lipogenic IgM did not bind to immobilized insulin (not shown). These results taken together indicate that the lipogenic activity could be assigned to IgM molecules and not to free insulin or insulin-insulin antibody complexes. The lipogenic IgM was

identified as anti-receptor antibody as it specifically competed with radiolabelled insulin for binding to insulin receptors. Table 2 illustrates that increasing amounts of IgM purified on an anti- μ-column increasingly inhibited the binding of insulin to the insulin receptors of adipocytes. Control IgM isolated from the serum of an IDDM patient without lipogenic activity had no effect on the binding of insulin to its receptor.

Normal human IgG stimulates lipogenesis in rat adipocytes in vitro (4). Although the IgM anti-receptor antibodies described here also caused lipogenesis, the two lipogenic effects differ with regard to both ligand and receptor. The stimulatory effect of IgG is exerted through the non-variable Fc portion of the molecule (4,5) and normal IgG does not compete with insulin for binding to the insulin receptor (P. Dandona, personal communication).

Table 1. Anti-insulin receptor antibodies in serum of IDDM patient are IgM

Treatment of serum	Serum fraction	% Relative lipogenesis of adipocytes
None	Whole	100
Anti- μ-column	Effluent (not IgM)	15
	Eluate (IgM)	75
Sephacryl 300	Large (IgM)	100
	Small (IgM)	0
Dissociation of complexes, Sephadex G-100	Large (Ig)	90
	Small (insulin)	0
Antibodies to insulin*	Whole	100
Antibodies to IgM!	Whole	5

Legend Table 1.

Sera were fractionated (2) using columns of goat antibodies to human μ-chain (Dako Immunoglobulins), bound to Sepharose (Pharmacia (1)), Sephacryl 300 (Pharmacia (12)) or Sephadex G-100 (Pharmacia). Antigen-antibody complexes were dissociated by acidification (0.01 M HCl, pH 2.7) of serum (2) before gel filtration. Control serum or fractions for each procedure were obtained from healthy donors and from an IDDM patient whose whole serum was negative for anti-receptor antibody activity. Lipogenic activity was computed relative to that found in 1 μl of the unfractionated serum (100%). Adipocytes were obtained from the epididymal fat pad of male Wistar rats (90-120 g) and the incorporation of D(U-^{14}C)glucose (4-7 mCi mol^{-1}; NEN) into lipid was measured as described previously (2,3). Maximal lipogenesis (100%) was equivalent to that produced by incubation of the adipocytes with insulin (10 ng ml^{-1}) and was 300% of control lipogenesis obtained without added insulin.

* Guinea pig anti-insulin antiserum (Miles-Yeda; titre 10^{-5}) was added (2 μl) to the lipogenic assay.

! Goat antiserum to IgM (Miles-Yeda) was added (30 μl) to the lipogenic assay.

Table 2. Purified IgM receptor antibody competes with insulin for binding to insulin receptors on rat adipocytes

	% Inhibition insulin binding to adipocytes	
Affinity-purified IgM (μg)	Origin of IgM	
	Test serum	Control serum
5	29	9
10	48	4
20	72	0

Legend Table 2.
Affinity-purified IgM eluted from an anti- μ column (see Table 1) was added in the indicated amounts to rat adipocytes (10^5 cells) in plastic tissue culture tubes containing 0.35 ml KRB buffer (pH 7.4)-0.3% bovine serum albumin and ^{125}I-insulin (35,000 c.p.m.). The tubes were incubated at 25°C for 40 min and the adipocytes were then separated from unbound insulin on a Millipore filter (EGWP, 0.2 μm), washed with ice-cold buffer and counted for radioactive content (13). Extent of binding was 1.7 pmol per 10^5 adipocytes of which 70% was specific (displaced by 1 μM cold insulin). Per cent inhibition was computed relative to the binding obtained in the absence of added IgM.

Table 3 documents the presence of anti-insulin receptor antibodies in the sera of IDDM patients before and after treatment with exogenous insulin. Ten out of 22 patients had these antibodies in their sera at the time they first presented, before they had been treated with exogenous insulin. We have had the opportunity to examine serial bleedings obtained after treatment of five patients who had been negative at the outset. Two of these patients became positive within 4 months of receiving treatment with exogenous insulin, one having been given human and the other porcine insulin.
The investigation described here was prompted by the observation that mice developed anti-insulin receptor antibodies spontaneously after immunization to insulin (2). These receptor antibodies were identified as anti-idiotypes to insulin antibodies, suggesting that they might have arisen as components of an idiotype-anti-idiotype network (6). The anti-idiotypes probably functioned as receptor antibodies by mimicking the conformation of the antigen insulin (7). We reasoned that humans might possibly develop similar anti-idiotypic insulin receptor antibodies in response to their own insulin antibodies produced by accidental immunization to exogenous insulin used for treatment. However, a large number of the pretreatment sera which we had believed would serve as negative controls were found to be positive for anti-receptor antibodies (Tabel 3). Therefore, we must conclude that autoimmunity to insulin receptors, rather than resulting merely from an iatrogenic accident, may be generated during the pathological process

intrinsic to IDDM. Moreover, the prevalence of these antibodies in an unselected group of patients suggests that their presence is neither sporadic nor infrequent (Table 2). We have no evidence to indicate whether or not the anti-insulin receptor antibodies in the IDDM patients are anti-idiotypes to insulin antibodies.

Table 3. Anti-insulin receptor antibodies in sera of IDDM patients before and during treatment with exogenous insulin

Serum donors	Anti-insulin receptor antibodies
Normal controls	0/20
IDDM patients	
Before treatment	10/22
After treatment	2/5

Anti-receptor antibodies in each serum were identified by two or more of the assays described in Table 1. Sera of 22 patients were obtained before they were treated with injections of insulin. Sera were obtained serially from five patients treated with injections of insulin after being negative for receptor antibodies before treatment. Two patients became positive for anti-receptor antibodies during 4 months of observation.

Insulin receptor antibodies have been found in a few dozens of patients with acanthosis nigricans and severe diabetes (8). It seems, however, that IDDM and the acanthosis nigricans diabetic syndrome are diverse entities with distinct anti-receptor antibodies. Unlike IDDM, the anti-receptor antibodies in the acanthosis nigricans patients are mostly IgG rather than IgM (8), the disease is extremely rare, the resistance to treatment with insulin is marked and the patients seem to have a primary structural defect of their insulin receptors (9).

Patients with IDDM, with or without IgM receptor antibodies, do

not have the degree of insulin resistance characteristic of the acanthosis nigricans syndrome. The disparate clinical entities associated with these anti-receptor antibodies may be attributed to the diverse biological effects of IgG and IgM receptor antibodies, to differences in the fine specificities of the receptor antibodies, to the intrinsic state of the insulin receptors and/or to the presence or absence of additional pathological processes.

Regardless of the mechanism of insulin receptor antibody generation, their presence indicates that autoimmunity in IDDM is not limited to islet-cells (1). The clinical consequences and theoretical implications of these anti-receptor antibodies may be important. What is their role, alone or together with viral infection and islet-cell antibodies, in the pathogenesis of IDDM? Do they influence the response to treatment or the development of late complications? Can they be used to identify degrees of risk or immune response genes? Are they a factor in the subclinical insulin resistance that is a prominent feature of IDDM (10)?

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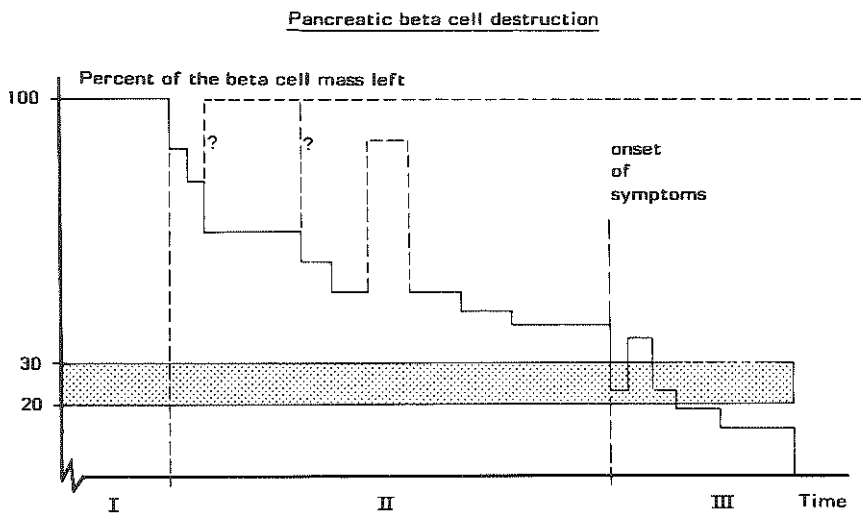
§5. Discussion

The etiology of childhood diabetes can be represented as pancreatic beta-cell destruction as a function of time (figure 2).

Fig. 2 Pancreatic beta-cell destruction



The gradual destruction of pancreatic beta-cell with time, clinical symptoms not developing before 70-80 percent of the original beta-cell mass has been destructed (paragraph 2). Question marks indicate putative phases of beta-cell regeneration. Roman numbers on the horizontal axis indicate time spans of:

I. genetic susceptibility (HLA-related)
II. subclinical expression of the disease (islet-cell antibodies)
III. the clinically overt state (when insulin is administered).

The papers presented in the previous paragraph can be assigned each to one the three phases of the time-course of childhood diabetes depicted in figure 2. The purpose of the following discussion is to present the findings in as much as they contribute to

the consideration of possible immuno-intervention of childhood diabetes.

This fascinating new field of clinical research has very recently received much attention, but only preliminary data are available (to be discussed in the final section of this paragraph).
To clarify the connection of the studies presented to this new field of research it must be understood that all investigations hitherto into immunosuppression of childhood diabetes dealt with possible prolongation of the remission-phase (paragraph 2), once the disease became clinically manifest. In other words: immunosuppressive measures were only tried in phase III of figure 2.

This discussion attempts to demonstrate that present findings in the other phases of the clinical course of childhood diabetes have consequences for the interpretation of the possible finding of prolongation of the remission-phase by virtue of immuno-suppressive measures, after insulin treatment is initiated. Furthermore new approaches are mentioned that will contribute to an improved understanding of each of the phases of the clinical course of childhood diabetes depicted in figure 2.

1. HLA-antigens as genetic markers for the susceptibility to childhood diabetes.

From twin studies it is known identical twins have a concordance rate of 50% or less (38,39). Gm-markers were not associated with diabetes-type-1a generally (paper 1), but they may still be in a small subgroup of children (paper 3), as far as the IgG-subclasses of their islet-cell-cytoplasmatic-antibodies are concerned.
Limiting this discussion to HLA:
From the multiplex families of paper 1 it was calculated that in our multiplex type-1a-diabetic families HLA-identity with an affected sib would carry a 11.6% risk for that sib to become diabetic himself. This is the same figure (12%) as calculated by Platz et al., who studied 51 multiplex young onset diabetic families and 212 monoplex families, meaning that only one sib had treated

diabetes (40). This figure is too low to employ HLA-identity as a practical guide for prospective studies finding children that later will develop diabetes, as will be demonstrated below.

Using this data the question can be asked: how many HLA-identical siblings (x) to newly diagnosed diabetic children should be followed to find 10 new cases in a two year period, if the ages of those siblings for practical reasons are limited from 10-19 years? This can be calculated from the following equation (41):

$$x = \frac{\text{number of new cases (10)}}{\text{(observation period (years))} \times \text{(corrected risk)}}$$

$$x = \frac{10 \times 100 \times 20}{2 \times 11.6 \times 1.60 \times 1.66} = 346$$

The corrected risk is arrived at as follows:

2 = duration of follow-up in years

$\frac{11.6}{100}$ = 11.6% = risk of development of diabetes in HLA-identical sib.

1.60 = increased risk for the age-cohort 10-19 years over that of the age-cohort of 0-19 years (chapter II - the ascertainment corrected annual incidences per 100.000 are 303 and 486, respectively.

1.66 = correction for the finding that one third of all siblings to diabetics become diabetic themselves within two years after their already diabetic sibling, according to a large study in England (42).

20 = correction to annual rates for the use of risks for the age-cohort of 0-19 years.

More than 300 HLA identical sibs would have to be identified to be followed prospectively for two years in order to detect 10 children of 0-19 years becoming clinically diabetic in that time-span. The ascertainment corrected incidence per year for that age-span in the Netherlands is 486 (Chapter II) and only one in four sibs will be HLA-identical as there is no evidence of selection against or for HLA-combinations in families with type-1-diabetes (5).
Thus, 346 x 4 = 1384 healthy siblings would have to be HLA-typed, which would involve 659 (1384 : 2.1 (the average dutch kindred)) families, which represents a formidable task against the background of the dutch total annual incidence.

In what way did the study of HLA by serology contribute to the understanding of the mechanism of the genetic susceptibility?

The mode of inheritance of susceptibility is complex and the "two or more gene concept", linked to HLA-DR3 and/or HLA-DR4 seems to fit the data best. This is in agreement with other studies, summarized by the late Dr. Andrew G. Cudworth et al. (5). They also described haplotype-clusters, involving genes over the whole of the major histocompatibility complex in association with susceptibility to type-1-diabetes. This finding suggests common ancestor haplotype-clusters among the patients, but, more importantly, lends support to the hypothesis of sets of interacting genes to act in concert within these clusters.

In another study (42), we found that the SB-locus, situated between the HLA-D locus and GLO (hence centromeric from HLA-D), shared no allotypes with type-1a-diabetes.
The cross-overs observed in our family-study, suggested that the HLA-A locus contributed more likely by linkage-disequilibrium to diabetic clusters than by truly being part of them. Thus, two loci situated between HLA-A and SB, were selected for their possible involvement, namely HLA-B and HLA-DR. Two haplotype-combinations, HLA-B8/HLA-DR3 and HLA-B15/HLA-DR4 were tested for third order disequilibrium with type-1a-diabetes. The indication found of the genes of two loci acting in concert, involving different classes of gene-products, namely HLA-B antigens (class I histocompatibility antigens) and HLA-D antigens (class II histocompatibility antigens), may have important consequences for the understanding of the immunologic pathogenesis and hence the consideration of future immunologic interventions. What these would be is at present unclear, as the interactions of the multiple antigens coded for by genes of the major histocompatibility complex (MHC) are only beginning to become elucidated.
The merit of the described suggestion of third order linkage-disequilibrium, arrived at by "classic" genetic analysis, is, that it can be tested by novel techniques, known as "fine genetics". This implies the use of DNA-probes on segments of chromosomes dissected by endonucleases.
Recently, D. Owerbach and associates at the Hagedorn Institute (43), described two such fragments, relevant for the association between HLA-DR4, the commonest haplotype in our childhood diabetic population, and insulin dependency. It is known class II-antigens (HLA-D related) show a marked genetic polymorphism associated with the beta-chains of these molecules (44). This polymorphism appears to be associated with several MHC genes, evidenced from hybridization studies (45). Thus, differences in hybridization pattern between DNA from healthy individuals and diabetic patients, using the beta-chain c DNA probe (p DR-β-1) after digestion with certain restriction endonucleases may be highly relevant. They found an increased frequency of one fragment among HLA-DR4 / HLA-DRx individuals as well as among HLA-DR4 / HLA-DR3

individuals, that were insulin dependent. They also described another fragment, occurring in 30-40% of controls, but in only 0-2% of the diabetics. Other probes, suitable for the examination of the HLA-B locus could yield similar results, perhaps supporting the proposed "two or more loci concept".

Should relevant DNA-fragments be found, monoclonal antibodies to their gene-products (membrane antigens) may be generated, facilitating the detection of cells carrying such antigens.

For the detection of children with a genetic risk for type-1a-diabetes, the accuracy using the above techniques could be higher than presently possible with serologic typing.

In what further way may the study of HLA contribute to better understanding of childhood diabetes?

The MHC-complex in humans (as well as genes of the H-2-complex in mice) encomprises a large variety of functions pertinent to presentations of type-1-diabetes.

With regard to insulin-dependent-diabetes it is relevant to mention biologic functions that have so far been associated with HLA-genes or their products: the HLA-antigens on the cell-membrane. Table 2 summarizes such functions, in mice (of the H-2-complex) and in humans (of the HLA-complex), contrasted to their possible relevance for all stages of childhood diabetes.

Table 2.

Function	Relevance for diabetes-type-1
1. Production of humoral antibodies	1. Islet-cell antibodies, thyroid, adrenal, parietal cell and other prevailing auto-antibodies (46). Antibodies to injected insulin.
2. Production of killer T-cells and helper T-cells	2. Pancreatic beta-cell cytotoxicity (47) Impaired suppressor T-cell function (48).
3. Regulation of immune response	3. Ibidem including mode of expression of autoimmunities.
4. Biosynthesis of some complement components	4. Ibidem
5. Regulation of embryogenesis	5. Unknown
6. Regulation of synthesis of some steroid hormones	6. Unknown
7. Regulation of ligand-membrane interactions of some protein hormones	7. Insulin action (49) also modified by insulin antigenicity.

With present serologic HLA-typing sub-groups of type-1a-diabetic patients cannot be separated easily in view of the already mentioned haplotype-clusters that contain genes in linkage to each other rather than to disease-states. Fine-genetics, referred to above, may detect and hence predict highly relevant entities within type-1a-diabetic patients. Such entities might include:

1. differences of islet-cell antibody occurrence in particular in pre-diabetic-states;
2. differences in velocity of loss of remnant endogenous insulin secretion;
3. differences in sensitivity to injected insulin.

2. Islet-cell antibodies as markers for childhood pre-diabetes

The second tool investigated for the evaluation of the predictability of diabetes-type-1a were antibodies to the islets of Langerhans (ICA). One human pancreatic specimen with previously established specificity and sensitivity for the assay of ICA was used for this purpose. The prevalence of ICA in newly diagnosed children was 80% with this system (paper 4), in the same order of magnitude of other studies, using fresh-frozen, rather than Bouin fixed pancreatic specimens. In controls, only 1% was weakly positive, indicating a better specificity in this respect than found in other studies.

Subsequently, the question was asked to what extent these ICA might be present before the diagnosis is made.

Two years before the study reprinted as paper 5 was started, the London group lead by the late Dr. A.G. Cudworth and Dr. G.F. Bottazzo set up a prospective study into the predictability of childhood diabetes by ICA. This study became known as the Barts-Windsor study. It involved 154 healthy brothers, 134 healthy sisters from 160 families having a diabetic child. ICA were assayed every 1-4 months with a mean follow-up of 2 (1.1-2.5) years at the time point the study reprinted as paper 4 was initiated. In their preliminary report (50) it was indicated six subjects of these families had developed insulin-dependency. Four of these six subjects had been ICA-positive from the moment they entered the study and had remained positive on repetitive testing until the disease became clinically manifest. The two other subjects had become positive during their follow-up. Of these six subjects two were HLA-identical sibs, two were haplo-identical sibs and two were parents.

In total, that study involved 473 healthy siblings and parents. In their final report (51) seven developed diabetes in two years and 54 of these subjects had ICA positivity without the development of clinical diabetes. If one assumes all these subjects (children and parents) would become diabetic eventually, the recurrence risk would be 54/473 = 11.2 %.

It is difficult, to compare this figure with the recurrence risk

derived from epidemiologic studies. Very few epidemiologic surveys have taken the recurrence risk in parents into account and only one corrected for incomplete ascertainment, the study from the Pittsburgh group (52). Another difficulty with the Barts-Windsor data is that several pancreas specimens were used for the assay of ICA and that the fluorescence was not expressed in titres.

Provided pancreatic tissue with defined sensitivity and precision for the detection of ICA would be used, this tool appears to have a high accuracy screening for pre-diabetic states in childhood (papers 3 and 4). A practical advantage of this method as an entry to identify pre-diabetes of childhood is that minute quantities of serum can be used. In positive cases, only 50 microliters of serum is needed for a reliable assay. As demonstrated in paper 3 and 4 the titres of prediabetic children as well as of newly diagnosed children were usually 4 or higher, if positive. Such quantities would be obtainable by fingerprick, a technique most families with diabetic children are used to, so that the participation of children younger than 10 years of age may be considered.

It should be noted that 20% of the newly diagnosed children did not have ICA. The one patient (paper 3) that developed ICA only after diagnosis did have 3+ islet-cell surface antibodies (ICSA) only, three years before her disease became manifest. This data and the sequence of the islet-cell antibodies developing in this particular patient, suggests that next to ICA, ICSA should be used as screening tools for prediabetes of childhood. For this method 1 milliliter of serum is presently required and the fluorescence assay involved is much more difficult.

At the moment we initiated our own survey of ICA-positivity among healthy sibs and parents of 22 multiplex families, we only knew of 4 children that had become diabetic in the Barts-Windsor study forementioned, with ICA positivity from the moment they entered that study. We agreed with our participants we would make the HLA-data available only to their family physicians, but would not disclose the - to us at that point uncertain - meaning of ICA-positivity in a healthy family member. We decided to look for ICA

in sibs and parents on the basis of single bleedings (with the above preliminary findings of the Barts-Windsor study on the persistence of ICA in mind). The 22 multiplex families, only 3 coming from our own clinic, participated voluntarily, in full knowledge of our own uncertainties to the meaning of HLA and/or ICA with respect to their future health.
With the data on the accuracy of predicting childhood diabetes by ICA (described in paper 3), one might feel less insecure about the prospect of a sib of a diabetic child having ICA without any signs or symptoms and this represents major problems. It was reported (the late Dr A.G. Cudworth, personal communication) that some participants of the Barts-Windsor study did resent their non-diabetic child becoming diabetic during those prospective studies. This experience holds an important warning if such studies are to be repeated. Participation of children not becoming diabetic may generate an undue awareness of the child and its family that the child may still become diabetic after an unknown number of years. The burden of this "partially inherited" life long disorder cannot be overestimated and causes numerous interactions in the families of children with treated diabetes (Chapter III - paragraph 1). Similar interactions may be projected by parents on healthy sibs by their mere participation in a screening program of childhood diabetes, that has no therapeutical consequences. Possible interference with the upbringing of these sibs cautions for a repeat of such studies on ethical grounds.
Before considering any screening program for pre-diabetes of childhood, more needs to be known on the nature of islet-cell-antibodies as predictors of childhood diabetes. Both islet-cell cytoplasmatic - and surface fluorescence-techniques are qualitative in nature, and the following three principle shortcomings (a-c) remain:

a.children with mumps infection (but not with Coxsackie, Measles, Rubella or Influenza) may have temporary ICA, not persisting for more than 2-4 months and without developing insulin-dependency subsequently (53). Beside this problem there are other limits to the immunologic specificity of ICA.

Islet-cell cytoplasmatic antibodies, usually not reacting with intact cells, are measured as islet-cell flurorescence on pancreatic slices. The antigenicity of the whole pancreatic beta-cell is assessed and the antigens of the whole islets they react with have not been specified to date. The complement fixing ICA appeared a fairly good screening agent for the pre-diabetic state of children. In view of the absent definition of their immunologic functions and biochemical nature they have been compared to "the crowd gathering around the accident". However, it happens with criminal accidents that "the criminals hide within this crowd to secretely enjoy the effects of their act" and the problem to identify them remains. Even the number of protein-isolates acting as antigens of the pancreatic islets was undefined until recently. S. Baekkeskov et al. (54) isolated two protein-fractions from cultured pancreatic islets, that reacted with a panel of ICA positive samples obtained from several institutions, including our own. They used detergent-solubilized radiolabelled islet-cell antigens from cultured human and rodent pancreatic islets, complexed these to antibodies isolated from the sera of diabetic children (obtained by plasmaphoresis) and detected the two reacting protein-fractions by gel electrophoresis after specific absorption to protein A-sepharose. Their findings that ICA reacted with at least a limited number of (two, thus detected) protein-fractions raises hopes to the further elucidation of the nature of these fractions.

Further, it is unknown <u>where</u> the relevant antigens reside within the islets. Studies with human-human hybridoma systems, where periferal lymphocytes of a diabetic child were fused with a human myeloma line, yielded one clone reactive with human pancreatic islets by fluorescence. Further analytical studies indicated that the target antigen was possibly situated in the cytosol of glucagon-, rather than insulin-producing cells of these islets (25). The significance of this experiment (the first to employ this novel technique with regard to childhood diabetes) remains unclear. ICA react with the cytoplasmatic contents of islet-cells as measured on fresh-frozen pancreatic sections. One would expect that cytotoxicity to intact islet-cells is mediated by islet-cell-sur-

face-antibodies (ICSA). These have been found to react with islet-cells of rodents. ICSA appeared to be organ-specific, but not species-specific. ICSA react mainly with pancreatic beta-cells and they can mediate a complement-dependent cytotoxic reaction (55). One would expect ICSA to react first with pancreatic beta-cells, destructing their membrane. Consequently the contents of the pancreatic beta-cells are exposed, which subsequently results in the generation of ICA. As forementioned, this sequence of events was actually documented in patient 2 of paper 3. In this view ICSA would be "the criminal" and ICA "his accomplice".

b. A second enigma, with possible immunogenetic implications, is exemplified by the detection of IgG-subclass restriction of ICA in a few diabetic children as described in paper 4. Early after diagnosis as well as in two pre-diabetic children (patients 1 and 2 of paper 4) mostly all four IgG-subclasses were present and no IgM-ICA was found. The absence of IgM-ICA suggests that auto-immunity towards the islets must have been going on for some time, because at the onset of auto-immune reactions antibodies of the IgM-class generally predominate. The presence of mostly all four IgG-subclasses early in the course of childhood diabetes suggests that there was no restriction of B-lymphocyte clones producing these multiple classes of antibodies. Of course, this absence of restriction does not exclude switching of such B-lymphocyte clones. Two children were found to have IgG_2-ICA restriction, despite relatively high (complement-fixing) ICA-titres. Admittedly, the demonstration of the IgG_2-subclass appeared to be more difficult than that of other IgG-subclasses. Hence, a dilution of 1:40 was used to demonstrate this subclass in ICA, as opposed to the dilution of 1:20 for the demonstration of the other subclasses, IgG_1, IgG_3 and IgG_4 (paper 4). These two children had "vertical transmission" of insulin dependency in their families. Although the family histories of these two children have not been compared to those of the other 42 studied with regard to IgG-ICA-subclasses, such family history is exception rather than rule in childhood diabetes. Although IgG-ICA-subclass restriction appears "a characteristic of an accomplice", this feature hints to a particular kind of "criminal act" in the comparison used before.

c. A third problem with ICA that needs to be considered is that ICA may encomprise idiotypic as well as anti-idiotypic antibodies against human islets. The relevance of this feature has been reviewed elegantly by C.H. Brogren and A. Lernmark (25): "The hybridoma technology provides a novel approach for investigating the network theory of Jerne, which implies that the antibody response is regulated through negative and positive feed-back mechanisms. Let us imagine that a monoclonal antibody able to induce diabetes has been isolated. According to Jerne's theory these antibodies, injected into syngeneic recipients will induce the formation of anti-antibodies (or so called anti-idiotype antibodies). We speculate that these anti-idiotype antibodies have a specific immunosuppressive effect, when administered to the diabetic recipient. A suppressive effect may be achieved by those anti-idiotypic antibodies depicting the antigenic determinants as the target molecules (internal image) which are expressed on islet-beta-cells. Anti-idiotype antibodies reactive with acetylcholine receptor antibodies have been produced after syngeneic mouse spleen cells were "educated" with acetylcholine receptors. Monoclonal anti-idiotype antibodies may thus prove to be useful reagents in studies of immune response regulation".
Human-human and human-mouse hybridomas have been produced from patients with auto-immune polyendocrine disease, including diabetes-type-1b (paragraph 1 of this chapter), thyreoiditis and sometimes adrenalitis (55). These hybridomas synthesize auto-antibodies that react with multiple normal human tissues, pituitary, gastric mucosa, endocrine pancreas and thyroid, at least partially explaining the multiorgan autoimmunity both in animals (25) and humans. The availability of large quantities of such monoclonal antibodies will aid in raising anti-idiotypic antibodies for the abovementioned purpose.
This specific example is relevant for "diabetes-type-1a" (paragraph 1) as well. In our own clinic (with presently 136 children of 0-19 years followed annually for 0-14 years, 4 years averaged) 10%-20% had antithyroid-, 4% anti-gastric-mucosa- and 1% anti-adrenal-antibodies, next to other varieties of auto-antibodies. If the organs involved in these cases could be demonstrated to be

antigenic by the sharing of common antigens to the pancreatic beta- cell, the generation of monoclonal anti-idiotypes may be useful to elucidate immune response regulation. The nature of this supposed common auto-antigen is still unknown. "Heterogeneity" of monoclonal antibodies, however, may still ensue, as these may react with still other constituents of the body, only sharing molecular substructures with relevant antigens.

A different problem with the interpretation of the significance of islet-cell antibodies deals not so much with their nature or specificity (a-c) but with their possible effects: it has been suggested immunoglobulins of diabetic children, that react with islet-cells, interfere with the islet's capacity to secrete insulin. In the presence of complement, the children's antibodies blocked the insulin release of rat islets (56). Cytotoxic antibodies markedly slowed down the insulin release of human islets in a system where the cultured islets were peri-fused (57). This effect casts doubt on tests assessing the secretory capacity of islet-cells as a measure of their (remnant) quantity: in the presence of islet-cell antibodies, interfering with insulin release, the C-peptide stimulation by for instance glucagon may well be hampered. During the first years of insulin treatment, when islet-cell antibodies prevail (paper 4), C-peptide reserve testing may "underestimate" the number of islets with functional potential, as this may be blocked by the potential of islet-cell antibodies abovementioned. This potential of islet-cell antibodies may seriously hamper the interpretion of beta-cell saving immunosuppressive therapies, reviewed in the last section of this paragraph.

Taken together, islet-cell antibodies in the course of childhood diabetes may become more heterogeneous as the disease-state progresses. Auto-antibodies towards islet-cells may or may not comprise reactivities with antigens that served as recipients for initial lesions of pancreatic beta-cells along the course of childhood diabetes: the heterogeneity of auto-antibodies in childhood diabetes is further expressed by the generation of an-

tibodies to insulin and to its receptor, discussed in the next section.

3. Insulin antigenicity

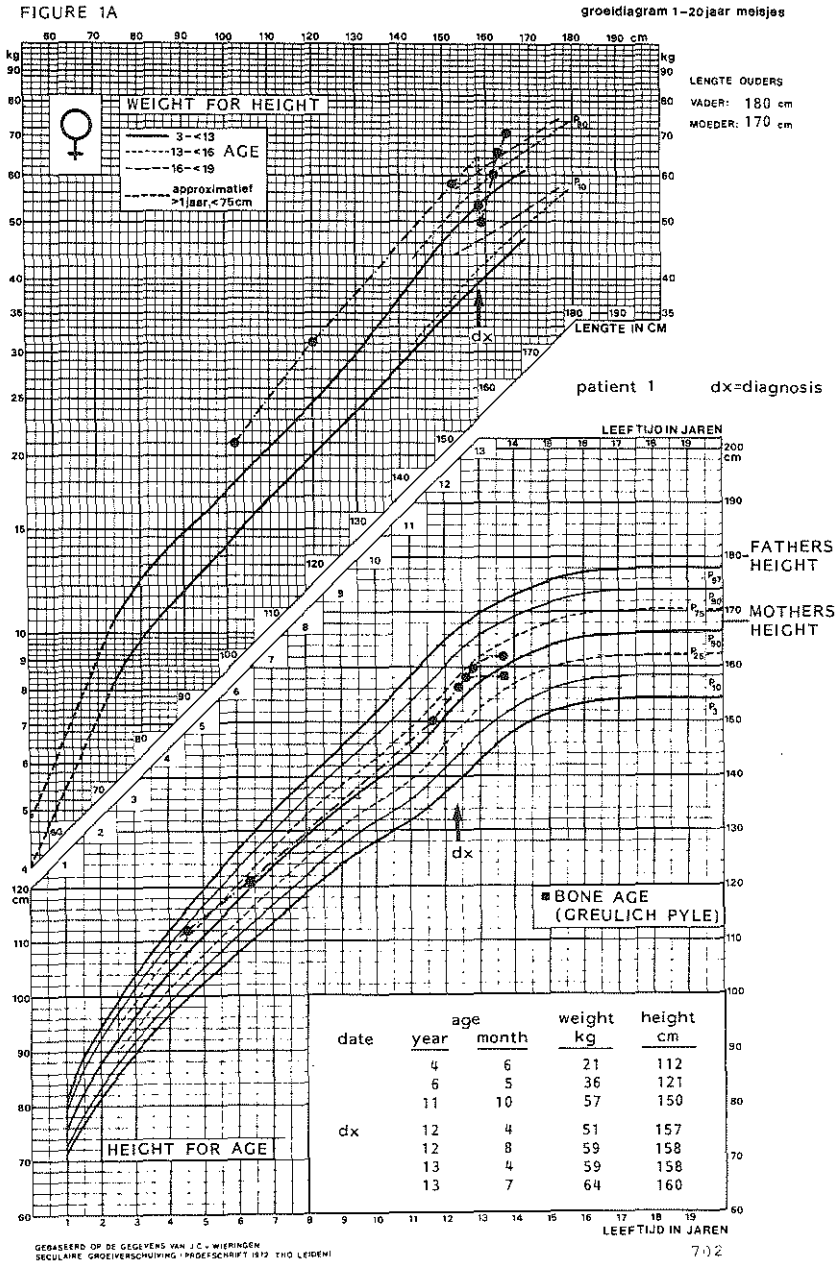
The one antigen that may be involved in the immunopathology of childhood diabetes and that has a known structure is insulin (paper 5). The concept that insulin antigenicity may be part of the disease process, evidenced by insulin-receptor-antibodies perhaps emerging as insulin-anti-idiotypes, suggests that the hormonal contents of damaged pancreatic beta-cells may also become involved in the auto-immunity of childhood diabetes.
Dr. W.G. Reeves very concisely summarized present knowledge on insulin antigenicity (58) as follows:
"The insulin molecule is of modest seize: 5600 daltons, the number of antigenic sites is limited, accordingly sera of treated patients have insulin-containing immune complexes of modest seize, which suggests that only one or at most two antibodies are able to combine with a single insulin molecule in these circumstances. Larger, precipitating complexes can be produced when bovine insulin is injected into guinea pigs: the insulins of these two species have 18 different sequences.
Insulins hitherto used for the treatment of human diabetics (bovine, porcine) show up to three amino-acid sequence differences, but the antibody produced in response to these injection is usually not specific for these variant residues, but reacts with determinants shared by the endogenous insulin molecule. Similar findings have been demonstrated in other species. Very little is known about the topography of these antigenic determinants on the surface of the molecule, but it is likely that they occur in sufficient proximity to each other to cause steric hindrance between the respective antibodies".
Next to species differences, the source of insulin preparations may be important: very highly purified porcine insulins and semi-synthetic human insulins are derived from pancreas extractions, r-DNA-insulins from E-coli pools. Minute but repetitive contaminations from any of these preparations with molecular forms other than native circulating insulin may still contribute to antibody formation after subcutaneous injection. Further it is of note that all insulin preparations for conventional treatment contain

complexed forms of native insulin, to slow down subcutaneous absorption. Complexed insulins may have different immunogenicity from crystalline insulin.
Many investigations have been done into the significance of antibodies to insulin in treated patients. Generally, these antibodies do not cause clinical insulin-resistance, but they can alter the molecule's pharmacokinetics (59).
Importantly, their presence may modify the duration of the remission-phase (60), further complicating the interpretation of prolongations thereof by virtue of immunosuppressive measures.
The insulin-receptor antibodies of the IgM-class described in paper 5, may or may not be related to the sub-clinical insulin resistance that is a prominent feature of treated insulin-dependent patients. It has been known for some time (61) that insulin-receptor antibodies have a paradoxical insulin-like effect on the metabolism of adipose tissue, namely lipogenesis. Therefore the bio-activity of the IgM insulin-receptor antibodies described was tested both in terms of lipogenic activity in the rat epidydimis fat-pad assay and in terms of specific displacement of radio-labelled insulin from isolated adipocytes (see paper 5 for methodology). It appeared relevant to search for clinical expressions of the IgM insulin-receptor antibodies in the two patients in which these IgM insulin-receptor antibodies emerged <u>after</u> the initiation of insulin therapy, compared to three patients in which these were not found. With regard to the ten of twenty-two children in which IgM lipogenic activity was found <u>before</u> insulin therapy: the specificity for insulin-receptors of their IgM has not been tested yet conclusively in insulin radioreceptor assays. The two patients referred to above, in which this specificity was documented (paper 5), are described next and are called patient 1 and patient 2.
Patient 1 is of negroid extraction, born in the Netherlands to parents from the Cape Verde-isles. At age 12.4, she was admitted with a severe keto-acidosis and dehydration with a history of thirst, hunger, weight loss of three weeks. She had been mildly obese as of age 4, her weight 2 kg above the 90th percentile for weight for height on the dutch growth chart (fig. 1a). Her health

history was otherwise unremarkable.
Before intravenous therapy was initiated her blood glucose was 32.2 mMol/l, glycosylated hemoglobin 18.6% (the presence of Hb-S and Hb-F were excluded), insulin < 1 mU/L. IgG-antibodies against the islet-cells were positive (titre 32) and fixed complement. Also titres of antibodies against mitochondria and smooth muscle were present but not against thyroid, adrenal or parietal cells. Her family history revealed no diabetes mellitus, both her parents had normal weights for height. Her HLA-D-genotype was HLA/DR3, homozygous.
During the first four months of treatment (fig. 2a) she used 0.7 - 0.9 U human semisynthetic human insulin/kg after initial stabilization also with human insulin (Monotard HM^R in two daily dosages). Between 3 and 6 months glucosuria did not exceed 30 grams per day, stable glycosylated hemoglobin varied from 7 - 9 %. A diet of 1500 cal. per day was prescribed, her weight stabilized during that same period at the 90th percentile (57 - 58.4 kg weight). Her chief complaint remained hunger and her weight increased until 70 kg two years later.
Patient 2 is of caucasoid extraction. She was 10 years old when her general practioner found glucosuria by coincidence. She was obese (like her mother). Between age 3 and 10 her weight had been 4 to 6 kg above the 90th percentile for weight for height on the dutch growth chart (fig. 1b). Her mother was also obese, but not her father. A 1000 cal. diet was prescribed without any other medication as her general practioner did not think of childhood diabetes. During the five months thereafter she lost 13 kg of weight and in the sixth month she developed mild complaints typical for insulin deficiency. Except for obesity, her health history was otherwise unremarkable.
A month later glucosuria increased until 75 gm/day, ketones appeared, and porcine insulin was administered (Mixtard^R in twice daily dosages) on referral to our hospital. During the second year of treatment her daily dosage was increased from 36 U to 44 U (fig. 2b).
Her family history revealed diabetes to be present in members of her fathers side: his mother used "tablets" as of age 69, her

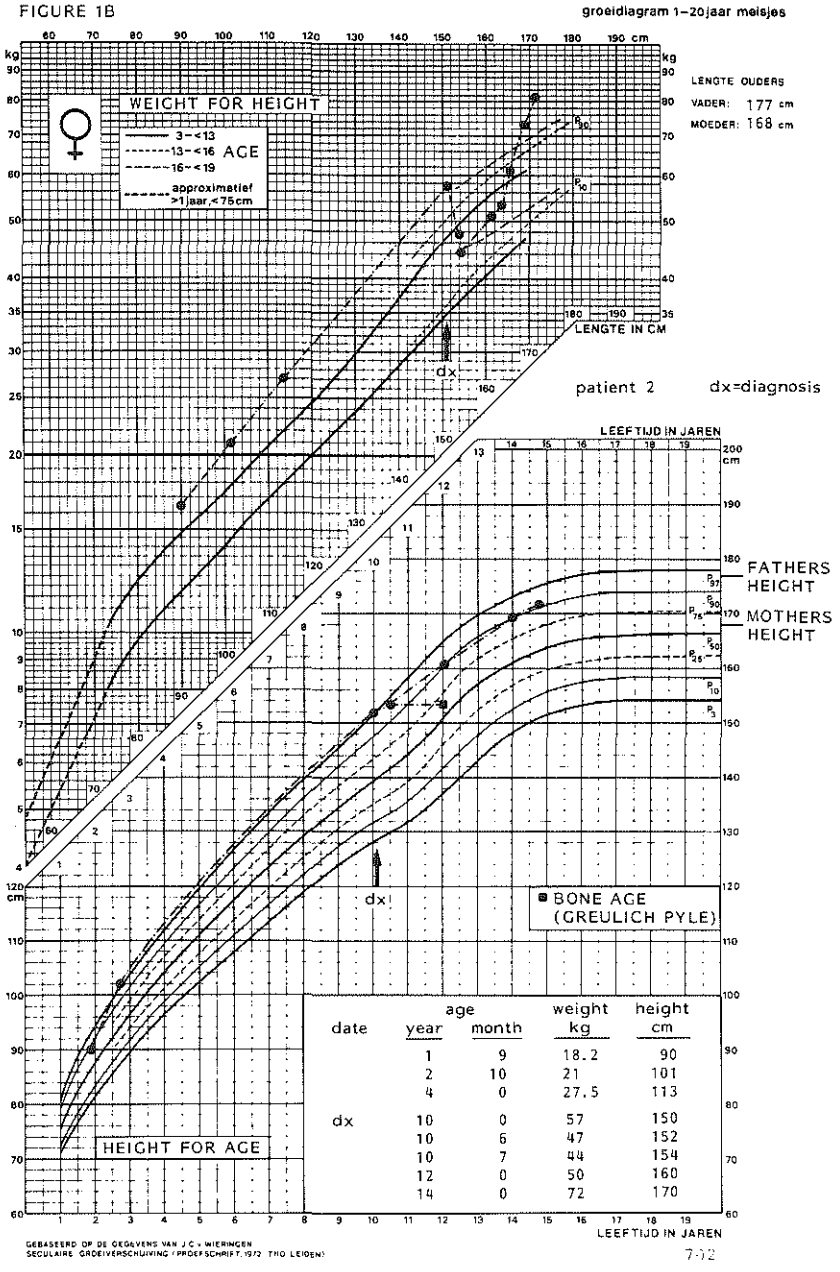
sister was insulin-dependent as of age 40, the son of this sister became diabetic at age 9. On the side of the mother the family history was unremarkable, except for obesity.
The patient's HLA-D-genotype was HLA-DR3/HLA-DR4, islet-cell antibodies were present (titre 8) without other relevant auto-antibody titres.
She was classified as a "slow-onset" diabetes-type-1a. In a series of 44 children she was the only child that had IgA-islet-cell antibodies next to IgG at the clinical onset of diabetes (paper 4). During the first eight months of treatment 0.4 U of insulin/kg was used, glucosuria did not exceed 30 gm per day and glycosylated hemoglobin did not exceed 10.2% (fig. 2b). No hypoglycemia was documented with a diet of 1500 - 2000 cal. per day and her weight increased from 45 to presently (44 months after the beginning of insulin therapy) 81 kg. Also her chief complaint remained hunger.
After one year of treatment islet-cell antibodies were still detectable (titre 4), after two years not any more.
Three other children, that had no significant (paper 5) lipogenic activities either before or up to one year after the initiation of insulin therapy, were two girls and one boy. They had no obesity beyond the 90th percentile for weight/height of figures 1a/1b, neither before or after the initiation of insulin therapy. (data not comprised in fig. 1a or fig. 1b).

FIGURE 1A



date	age year	age month	weight kg	height cm
	4	6	21	112
	6	5	36	121
	11	10	57	150
dx	12	4	51	157
	12	8	59	158
	13	4	59	158
	13	7	64	160

FIGURE 1B



date	age year	age month	weight kg	height cm
	1	9	18.2	90
	2	10	21	101
	4	0	27.5	113
dx	10	0	57	150
	10	6	47	152
	10	7	44	154
	12	0	50	160
	14	0	72	170

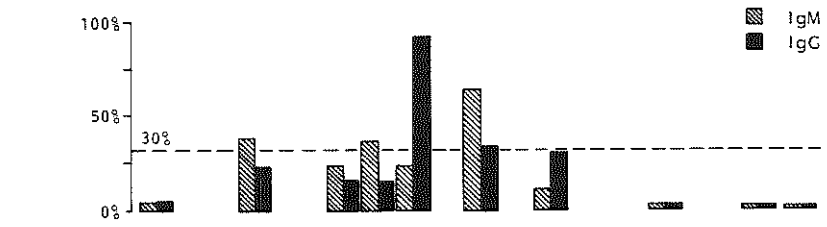
The time-course of patient 1 and patient 2 is depicted in figure 2a and figure 2b, respectively. Apart from the lipogenic activity of the IgM insulin-receptor antibodies, the lipogenic bio-activity of the IgG isolated from their sera was plotted. One reason for doing so was the recent observation of Khoker and Dandona (personal communication) that human IgG has a powerful lipogenic effect by itself (also see paper 5).

Patient 1 developed high lipogenic activities, first IgM, subsequently IgG before high affinity "classic" insulin antibodies had been detected, kindly measured by Prof. Dr. J.H.H. Thijssen of the Endocrine Chemistry Department of the Utrecht University School of Medicine. In that time period her insulin dosage (36 U per day) was lower than before and after and also her glycosylated hemoglobin levels were lowest then. Her urinary C-peptide excretion as well as her C-peptide reserve suggested remnant endogenous insulin secretion, synchronous with a temporary reduction in insulin requirements.

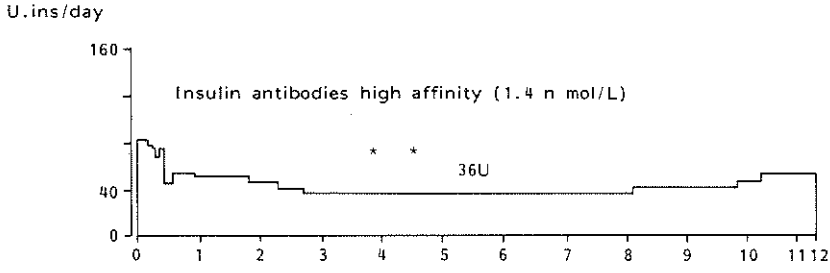
Patient 2 developed also high lipogenic activities, but in her these consisted primarily of IgM up to a year, before high affinity insulin antibodies were detected. In this time period her insulin was gradually increased until 50 U per day, but, in contrast to patient 1, her stable glycosylated hemoglobins were never lower than 11.2%. Her weight increased from 45.5 until 74.6 kg, presently (5 years after the beginning of insulin therapy) 81 kg. Her urinary C-peptide was lower from the onset of insulin treatment, compatible with the absence of a temporary remission in insulin requirements.

As illustrated in figure 1a and figure 1b both patient 1 and 2 had previous obesity prior to the onset of type-1-diabetes, in contrast to five other type-1-diabetic children of the same age, not depicted in these figures.

Figure 2a TIME-COURSE PATIENT 1

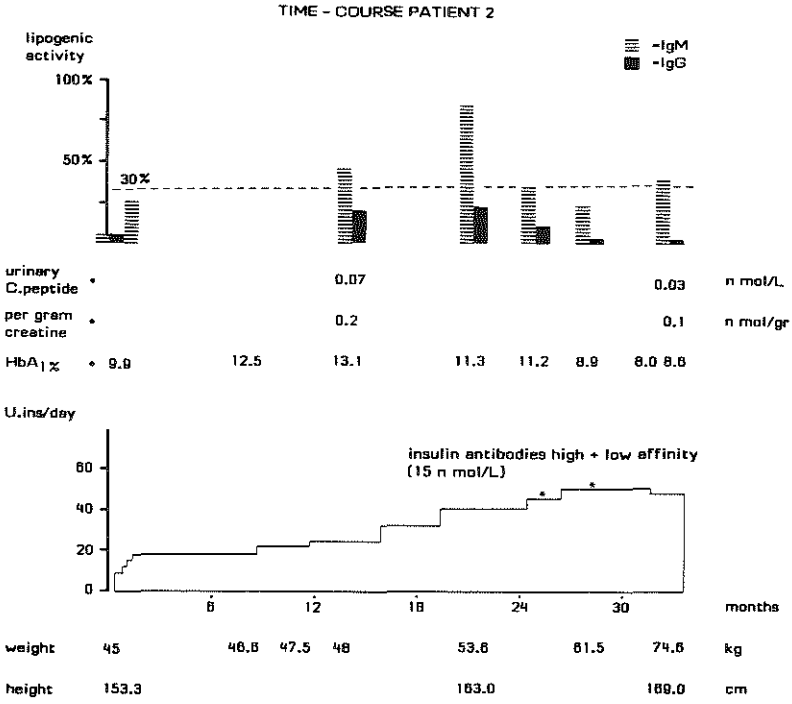


urinary C.peptide	0.27	0.80				0.42		0.26	0.28	n mol/L
per gram creatine	2.4	11.7				9.9		1.9	2.1	n mol/gr
$HbA_{1\%}$	14.1	10.2	8.4	6.8	8.7	10.7	10.1	12.3	10.6	



weight	50.2	56.0	57.0	58.7	58.4	57.5	57.0	58.5	66.0	kg
height	157.7							161	162	cm

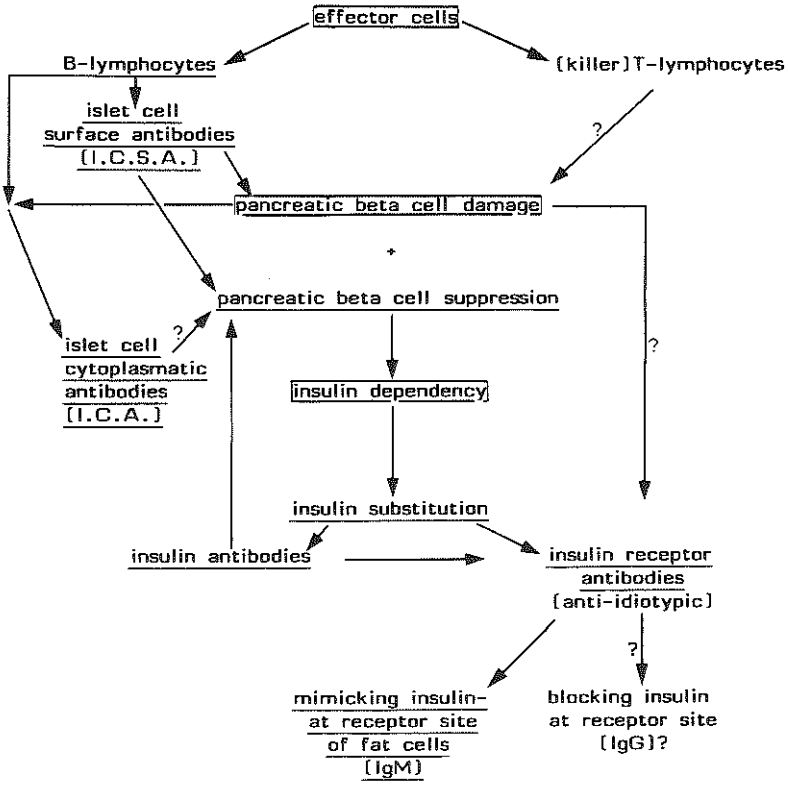
Figure 2B


TIME - COURSE PATIENT 2
lipogenic activity
100%
50%
30%
-IgM
-IgG
urinary C.peptide
0.07
0.03
n mol/L
per gram creatine
0.2
0.1
n mol/gr
HbA1 %
9.9
12.5
13.1
11.3
11.2
8.9
8.0 8.6
U.ins/day
insulin antibodies high + low affinity (15 n mol/L)
60
40
20
0
6
12
18
24
30
months
weight
45
46.6
47.5
48
53.6
61.5
74.6
kg
height
153.3
163.0
169.0
cm

These preliminary observations have clinical and pathophysiological implications with respect to the role of immunoglobulins in the regulation of the metabolism of adipose tissue.
Clinically, these activities may be related to the experience that degrees of obesity in treated childhood diabetes are not a simple function of insulin dosages, diet and/or physical exercise, but that body weight attained under treatment may also be mediated by these immunoglobulins. This is in keeping with the clinical experience that it may be very difficult in very obese adolescent girls (as patients 1 and 2) to loose weight by changes in the usual therapeutic regimen of their diabetes. Pathophysiologically, the significance of the IgM insulin-receptor antibodies was discussed in paper 5. The lipogenic activity of IgG, that appeared to predominate in patient 1 over that of IgM, may be related to the non-suppressible insulin-like-activity-P, described by Froesch and colleagues to account for 90% of the insulin-like activity of human serum. NSILA-P has a molecular weight of about 150,000 Daltons like human IgG, whereas IgM is a pentamer, with a molecular weight of about 900,000 Daltons. The lipogenic effect of human IgG appeared to be mediated by the (non-variable) F_c part of the IgG molecule and was not exerted via the insulin receptors of fat-cells (62). On a recent symposium (63) Khoker, Taylor and Dandona expressed the need for a critical reassessment of the observations that 1) insulin-receptor antibodies induce a paradoxical stimulation of adipocytes in vitro; 2) their finding that human IgG stimulated adipocytes in vitro; 3) the findings of paper 5, that IgM isolated from newly diagnosed diabetic children contained specific insulin receptor antibodies, that might be anti-idiotypic antibodies to the insulin injected.
The present observation of clinically expressed obesity in patients 1 and 2 implies that the sera of treated diabetic children may be very useful for such critical reassessment.
The findings further exemplify the complexity of functions of (auto-)antibodies involved in early stages of childhood diabetes, either emerging spontaneously or as a consequence of insulin injections, depicted in figure 3.

Figure 3.

The etiology of childhood diabetes mellitus, a schematic representation of findings (arrows) and concepts (question marks) of the roles of humoral antibodies.



4. Immuno-intervention in childhood diabetes

At the beginning of this discussion it was noted that centres have recently begun to investigate the effects of immuno-suppression in newly diagnosed patients. The important animal experiments (71) that lead to these clinical trials will not be reviewed here. Table 3 summarizes the methods used in humans and their possible side-effects.

Table 3. Studies of immuno-suppression in childhood diabetes.

Immuno-suppression used.	Possible side-effects.
Plasmapheresis (Ludvigsson, Sweden) (64)	thrombosis, hepatitis.
Prednisone (Ellis, New Zealand) (65)	growth retardation insulin-resistance, a.o.
Cyclosporin A (Stiller, Canada) (66)	hirsutism, gingival hypertrophy, anemia, hepato-/nephro-toxicity.
Monoclonal anti-T cell antibody-T_{12} (Eisenbarth, U.S.A.) (67)	mild transient rash anaphylaxis?

The effects of immuno-suppression in the clinically manifest disease were expressed as prolongations of the remission in insulin requirements after the initiation of insulin therapy. Also, the preservation of the capacity to secrete insulin endogenously has been assessed. Prednisolon was also tried on a pilot basis in limited numbers of children with disappointing results after two years of follow-up (68). With regard to the other means of

immuno-suppression abovementioned, formal studies are underway and the results mentioned here are preliminary. It should also be mentioned most trials involve older children and young adults.
Stiller et al (66) used cyclosporin in a dosage of 10 mg per kg per day, adjusted to serum levels ranging from 100-200 ng per ml and found considerable side-effects. At the time of last observation (48-114 days, the cyclosporin initiated within 6 weeks after diagnosis) three of seven patients were receiving no insulin.
In another group of patients, initiated on cyclosporin two or more months after diagnosis, none had a reduction in insulin dosage of 50% or more and there was no significant increase in C-peptide levels.
Eisenbarth et al. (67) using monoclonal T_{12} antiserum in a similar study, concluded that none of the pilot studies on immunotherapy of type-1-diabetes so far gave clear beneficial results.
In other studies (without applying immunosuppression) intensified conventional treatment during the first year of disease was associated with prolongation of remission-phase (14), although not as markedly as seen in the patients referred to above.
At present, the preliminary findings on the effects of immunosuppression raise the following questions:

1. why did some patients appear to react much more favorably than others?
2. to what extend was the prolongation of the remission-phase due to the immunosuppression applied?
3. what relevant humoral antibodies were suppressed?

Some pathophysiological difficulties interpreting the clinical phenomenon of a remission-phase were discussed in the previous sections.
It seems to the author these important questions can only be answered clinically by previous stratification of patients, with regard to already known characteristics of remission-phases in sub-groups of patients. Such stratification would include controlling for the following factors:

1. the age of the patients as young children (below age 5 in particular) as these very young children have shorter remission-phases (if any) compared to their older peers on conventional

treatment (69);

2. the quality of metabolic control during the follow-up of trials;
3. velocity and ferocity of pancreatic beta-cell loss prior to diagnosis.

The weight of the first two variables, with respect to their influence on remission-phases, may be established by age selection and by choosing optimal treatment-modalities in defined age-groups. Having done so, it may be less difficult to evaluate effects on remission-phases by virtue of immunosuppression.

The third factor is dictated by circumstances prevailing on referral of a newly diagnosed child and can only be controlled for by screening programs of pre-diabetic states of childhood diabetes. One might envisage the identification of children at risk by "refined" HLA-typing, defined islet-cell antibodies, followed by intensive treatment alone or in combination with immunosuppressive therapy in a randomized prospective study, as soon as "early phase" glucose-intolerance (70) developed. The ethical legitimicity of such a trial, that has potential therapeutic consequences, should be discussed with platforms of parties involved, notably ethical committees of medical faculties and patient organizations.

In view of the daunting prospects of children suffering from the disease, it is hoped for that such trials can be considered in the not too distant future: along with expected progress in screening methodology, improved specificity of immuno-intervention and defined means to assess its effects.

6. Summary

The findings reported in this chapter may be summarized as follows:

1. The strong association between childhood diabetes and HLA-DR3/HLA-DR4 is confirmed.
2. The recurrence risk for a sib of a diabetic child being HLA-identical to that child is about 12%.
3. The susceptibility to childhood diabetes may be mediated by two or more loci, one associated with HLA-B8/HLA-DR3, the other with HLA-B15/HLA-DR4, in third order linkage disequilibrium.
4. Generally, childhood diabetes is not associated with Gm-markers.
5. Absence of cytoplasmatic islet-cell antibodies at the clinical onset of the disease occurs in 20% and absence of either HLA-DR3 or HLA-DR4 in less than 10%. Absence of these immunologic parameters may be found in unusual syndromes with childhood insulin-dependency.
6. The accuracy of islet-cell antibodies, measured by immunofluorescence, to detect pre-diabetic states in childhood is probably high, although their pathogenetic significance is unclear.
7. Restriction to single IgG-subclasses in islet-cell fluorescence suggests linkage to Gm-markers for the small subgroup of diabetic children in which this was found, with regard to the expression of this auto-antibody.
8. Besides islet-cell autoimmunity, autoimmunity towards fat-cell insulin receptors may develop spontaneously.
9. IgM insulin-receptor antibodies arising after treatment with insulin may be associated with obesity.
10. Before the consideration of "immunoprevention" of childhood diabetes, more needs to be known about the pathophysiology and the immunology of the disease and disease states in which immuno-intervention is applied.

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CHAPTER II EPIDEMIOLOGY

"for Art and Science cannot exist
but in minutely organized Particulars"
(Blake)

§1. Introduction

The fourth year medical student lectures at the Erasmus University, taught by Prof. Dr. H.A. Valkenburg and his associates contain a quotation from Francis (1954):
"Epidemiology provides a third dimension to the understanding of disease by creating awareness of the nature of the environment in which disability arises, of the factors in the community which contribute to its causation and, in turn, its effect upon the community".
The retrospective investigation of the incidence of childhood diabetes in the Netherlands (paragraph 2), was the result of a collaborative effort of the "Working Committee on the Epidemiology of childhood diabetes" established in 1978 through the initiative of Prof. Dr. W.H.H. Tegelaers, chairman of the Department of Pediatrics at the University of Amsterdam Medical School.*
With regard to the methods of epidemiologic studies it is interesting to review the minutes of the committee: first, the testing for urinary glucose in healthy school children was considered as a method to detect children in which insulin-deficiency might be developing. A pilot-study was carried out by two school-physicians M. Wirix-Nagelsmit and J.M. van der Ros, demonstrating nil positi ves among 450 healthy children.

* The members to the working committee, other than the authors of the paper reprinted, in alphabetic order, were:
Prof. Dr. J.L. van den Brande, Dr. H.F. Dankmeyer, Dr. M.H. Gons, Drs. M. Jansen, Prof. Dr. G.A. de Jonge, Prof. Dr. W.H.H. Tegelaers, Dr. H.P. Verbrugge, Drs. A.A.M. Vloemans and Drs. F. Wafelbakker.

This approach was prompted by the clinical observation, impression rather, that relatively more children, compared to former years, presented with asymptomatic glucosuria, when first seen clinically, than with keto-acidosis. Indeed, in our own hospital we found in retrospect 12 (10-20) percent of 55 newly diagnosed children with diabetes mellitus presented with keto-acidosis (blood pH $<$ 7.2.) between 1975-1980, whereas 36 (30-40) percent of 71 newly diagnosed children did so between 1970-1975 (1).
The rejection of urinary glucose as marker for beginning diabetes of childhood coïncided with the formal acceptance (1979) of diabetes-type-2 being different from diabetes-type-1 by authorities as the NIH and the WHO: in diabetes-type-2 glucosuria may be a marker by virtue of its slow onset; in diabetes-type-1 (childhood diabetes) the clinical onset is much more sudden, so that glycosuria would only be present weeks or months at most before its onset (Chapter I - paragraph 1).
Subsequently this approach was abandoned and a retrospective analysis of newly diagnosed children was considered by the committee of which the author of this thesis had the honour to become chairman.
The first question raised was what criteria should be used for diabetes-type-1 in childhood.
In the epidemiologic sense, it is an advantage of diabetes in childhood that patients are either insulin dependent or not. Temporary use of insulin is occasionally seen in neonates (2), but such cases can be excluded by checking on reported cases of neonatal diabetes, expected to be rare. In contrast to type-2-diabetes, the incidental administration of insulin, for example around operations in elderly patients, does not occur in youth.
Mild glucose intolerance, usually not accompanied by glucosuria, may be seen in obese (pre-) adolescents, but these children never require insulin (3), nor do most of the genetically determined mild cases referred to in paragraph 1 of chapter I.
It is of course possible that children with mild glucose-intolerance are inadvertently treated with insulin. In such case one would suppose that either hypoglycemia would develop or that such low dosages were needed that specialists - who invariably treat

children with insulin dependency in the Netherlands - would doubt the necessity thereof. In incidence studies, assessing newly diagnosed children retrospectively only, the contribution of such cases was felt to be low, if any.
One exception should be made: the insulin-dependency that concurs in up to 8 percent of the patients with cystic fibrosis (4). Interestingly, the HLA-DR3 and HLA-DR4 haplotypes predominated markedly in the cystic fibrosis patients on insulin over cystic fibrosis patients without fasting hyperglycemia (5). Up to 8 percent of all living cystic fibrosis patients under the age of 19 years in the Netherlands would represent 80 or less cases (Dr. L.P. ten Kate, Institute for Anthropogenetics, University of Groningen, personal communication). Compared to an expected prevalence of 4,000 for all minors under age 19 with insulin-dependency this subgroup of children with cystic fibrosis would represent too small a fraction to be considered separately.
With these considerations in mind it was felt "insulin-dependency" as a criterion would encomprise "classic" type-1-diabetic children. The committee decided to conduct a retrospective survey of the incidence of childhood diabetes under age 19 all over the Netherlands, using this simple, but practical criterion, by a questionnaire to all dutch pediatricians and internists.
The second question raised was how to ascertain the data obtained from a retrospective survey among dutch pediatricians and internists.
Methods used in Scandinavian countries (drug-registry or hospital-file checking) were either impossible or very difficult in the dutch health-care system. On the other hand the Netherlands are unique in having the largest relative membership of the Dutch Diabetes Association (Diabetes Vereniging Nederland - D.V.N.) in the world, with 35.000 members in a country of 14.3 million inhabitants. With the help of the medical advisor to the D.V.N., Dr. H.F. Dankmeyer, it was decided to separately conduct a retrospective survey on the incidence of childhood diabetes by a questionnaire to the members of the D.V.N., as an ascertainment procedure.
The report of the investigation, conducted by the Netherlands In-

stitute for Preventive Health Care under direction of Dr. G.J. Vaandrager, is reprinted in the next paragraph.
Other data forthcoming of this epidemiologic study were published in a monograph in the dutch language ("De incidentie van insuline-afhankelijke diabetes mellitus bij 0-19 jarigen in Nederland 1978-1980" - G.J. Vaandrager, F.J. Veenhof, M.M. van der Klaauw - published by the Nederlands Instituut voor Preventieve Gezondheidszorg (NIPG-TNO) Leiden, 1984).

§2. Addendum

The incidence of childhood diabetes in the Netherlands. A decrease from north to south over north-western Europe?

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Introduction

In the past decade incidence-rates of childhood diabetes (0-14 or 0-19 years of age) were determined in all Scandinavian countries (1-4) and parts of the United Kingdom (5). In these studies high percentages of ascertainment were possible by virtue of the health-care system: drug-registry and hospital files could be used. In the Netherlands there is no nationalized health-care system, therefore such methods for ascertainment cannot be used. However there is an effective registration system. We decided to conduct a retrospective survey on the incidence of insulin dependency among all diabetic children in the Netherlands from 0-19 years of age, during 1978, 1979 and 1980.
The Netherlands national register counted 4.46 million youngsters from 0-19 years at that time, of whom 6.1 percent were born to first generation immigrants. In the health-care system only paediatricians and internists would treat insulin dependency in minors and both types of specialists act as registered consultants.

Methods

A questionnaire was sent to all consultant paediatricians and consultant internists to obtain data on newly diagnosed children between 1978 and 1981. The questionnaire contained only questions regarding the initials of the child's name, sex, age, date of the first insulin injection and residence at that time (no attempt was made to identify children of immigrants or of non-caucasoid background). The same information was obtained separately from the Dutch Diabetes Association, to ascertain the data obtained from the questionnaire sent to the specialists.

The questionnaire was sent to the 660 consultant internists and the 263 consultant paediatricians in the 181 general hospitals and to a contact person in each of the 8 internal medicine and 7 paediatric university clinics, including residential care centers. After one or two calls 100 percent of the paediatricians and residential care centres responded between February and September 1981.

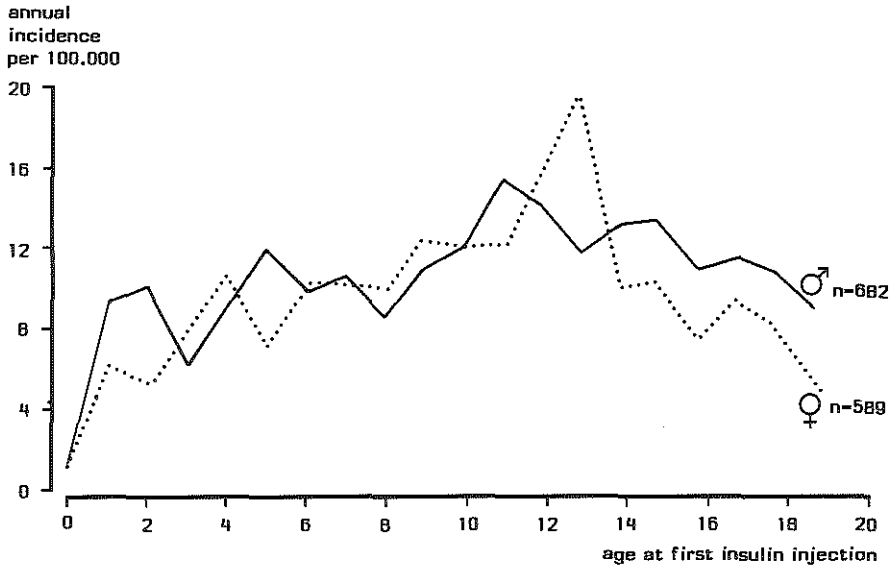
Patient-members of the Diabetes Association were requested to submit the same information after a call to do so in their monthly periodical, sent to all 35.000 members, 2.44 per 1000 inhabitants, with a range from 1.87-3.26 per thousand over the eleven provinces in the Netherlands. The membership of the Diabetes Association is high as members can obtain treatment materials for retail prices at local offices of the Association. The request to the members was published at a time point that 80 percent of the specialist responses had been obtained. In addition to the above mentioned questions the members were also asked who was their child's doctor: all answers contained either a consultant paediatrician or a consultant internist, confirming the premise that all diabetic children were treated by either one of these consultant specialists. Also, all treating doctors thus reported were present on our mailing lists.

The paediatricians reported 856 children, the internists 415, together 1271 youngsters from 0-19 years of age in whom insulin substitution was initiated and continued in 1978-1980.

From the Diabetes Association 305 complete responses were obtained of which 268 could be found also in the specialist's responses. This implied a reporting percentage of 21 from the Diabetes Association and an ascertainment percentage of 88 of the total number of patients found by the questionnaire among the specialists. For 1978 the ascertainment percentage was 85 percent, for 1979 84 percent and for 1980 93 percent, not statistically different. The method used for the estimation of the corrected number of new patients and its confidence limites (expressed as S_D in table 2) was based on the capture-recapture census described by Bishop et al. (6) and by Sekar and Deming (7). A premise with this method is that the response of the patients/members of the Diabetes Association was independent of that of the specialists. The homogeneity of each file (physicians and patient/members of the Diabetes Association) was tested by comparing ascertainment percentages found for answers with regard to age, sex, kind of specialist, grade of urbanization (8) of living place, month of the first insulin injection and province of living at that time. The two-sided chi-square test was used, no differences were found below the 5 percent level except for "age" and "kind of specialist". If however a distinction was made between paediatricians and internists this relation was no longer present: both the degree of completeness of the files and the age of the patients were related to the factor "kind of specialist". The ascertainment of the paediatricians' data (94 percent) was considerably higher than that of the internists (75 percent) (table 1). For the age-groups 0-4, 5-9, 10-14 and 15-19 the paediatricians ascertainment percentages were respectively 98, 90, 97 and 100 percent, of the internists -, 67, 70 and 77 percent. The material was split-up according to the two subgroups of specialists. The incidence rate was then calculated: the two sets of data were multiplied by the reciproce of their ascertainment percentages and subsequently added. Statistical calculations for geographic distributions and for degree of urbanization were done by the two-sided chi-square test, deviations from uniform distribution over the months of the year were tested by a modification of the

Kolmogorov/Smirnov test (9). The level of significance chosen was $p < 0.05$.

Figure 1



Results

Figure 1 depicts the incidence by age at the first insulin injection, separated for the two sexes. The male to female sex ratio's in the different age groups were: 0-4 years: 1.18, 5-9: 1.18, 10-14: 0.94, 15-19: 1.34, for 0-14: 1.05 and 0-19: 1.12.

Table 1 shows the ascertainment percentages were 94 percent for paediatricians and 75 percent for internists, while this percentage decreased also with age: a significant ($p < 0.02$) progressive decrease was found between five year intervals.

Table 1

Insulin dependency reported by type of specialist, patient/members of the Diabetes Association, age in years and ascertainment percentage.

	Specialist	Patient/ members	Both files	Ascertainment percentage	
Paediatrician	856	206	194	94	$p<0.001$
Internist	415	99	74	75	
0- 4	179	50	49	98	$p<0.02$
5- 9	323	90	80	89	
10-14	463	99	87	88	$p<0.02$
15-19	306	66	52	79	

Table 2 indicates the incidence rates found with a distinction between sex and age, and the standard deviation (S_D) of the method of estimation. The ascertainment corrected incidence was 10.95/100.000 per year for 0-19 years and 11.10/100.000 per year for 0-14 years. The 95 percent confidence intervals were 10.47-11.43 and 10.64-11.56 respectively for these age groups.

Table 2. The three-year incidence and the incidence per 100.000 inhabitants per year according to sex and to age group (1978-1980); after correction for ascertainment.

sex age (years)	number	S_D	mean population per year (Central Bureau for Statistics, 1979-1982)	incidence per 100.000/yr	S_D
boys					
0- 4	102	3.4	456.327	7.45	0.25
5- 9	202	11.9	564.636	11.91	0.70
10-14	261	11.2	625.878	13.88	0.60
15-19	228	16.5	633.133	11.98	0.87
0-19	790	22.8	2.279.974	11.55	0.33
girls					
0- 4	82	(-)[1]	434.746	6.29	(-)[1]
5- 9	164	5.9	539.960	10.11	0.36
10-14	266	15.9	597.359	14.84	0.89
15-19	163	16.8	605.746	8.95	0.92
0-19	676	24.3	2.177.811	10.35	0.37

[1] Ascertainment 100 %

The range of the differences between the Netherlands provinces was 8.9-13.7/100.000 children from 0-19 years of age. These differences were not statistically significant.
"Countryside", "urbanized municipalities" and "large municipalities" had incidence rates of respectively 11.61, 10.52 and 11.14/100.000 per year, not significantly different.
The distribution of the months of the year in which the first insulin injection was administered revealed no statistically significant differences for 0-4 nor for 5-9 year old children. In the

age groups 10-14 and 15-19 years a seasonal influence was present with the incidence rates higher in the winter months than in the summer months ($p < 0.05$ respectively $p < 0.01$, for these age groups). When the seasonal varation was studied for the three years separately, these differences were significant for 1978 and 1980, but not for 1979.

Discussion

The annual incidence found in the present study over the whole of the Netherlands (14.3 million inhabitants) was lower than that found in any other ascertained survey in western Europe conducted in the same time period. The trends in age of initiation of insulin treatment and the distribution over the sexes were very similar to those found in other studies. The present survey found no seasonal variation in the onset of clinical diabetes in children younger than 10 years of age. No significant differences in incidence were found with regard to the distribution over the provinces of the Netherlands, neither in the degree of urbanization. This latter finding is perhaps not surprizing as truely rural areas are rare in the densely populated Netherlands.
Given the limits of the Netherlands health-care system to employ drug-registry for ascertaining the data, the capture-recapture census method was chosen to calculate the confidence limits of the incidence found by two separate questionnaires. The advantage being that the largest childhood population so far could be examined relatively simply, the disadvantage being that an ascertainment percentage of only 79 percent was found for the children aged 15-19 years. For children younger than 14 years of age almost all responses were obtained before the survey among members of the Dutch Diabetes Association was initiated an the ascertainment percentage for that age-cohort was 94 percent. It is likely that the difference in ascertainment percentages found between paediatricians and internists was associated with the much higher "density" of newly diagnosed children seen by paediatricians than by internists. Over the three years of the study 3.25 cases were per reporting paediatrician (856/263) and 0.62 cases per inter-

nist (415/660).

It was found that 837 newly diagnosed children from 0-14 years were reported by consultant paediatricians (94 percent) and 287 children from 14-19 years by consultant internists (87 percent). These percentages indicate a referral pattern sharply distinct by the age of the newly diagnosed diabetic children in the dutch health-care system.

The incidence found for children 0-14 years of age was almost threefold lower than that found in Finland (1) almost two-fold than in Sweden (2) or Norway (3) and also lower than that reported from Denmark, excepting children from 0-5 years old (4). As emphasized by Christau et al. (10) it is very difficult to compare incidence rates even with high percentages of ascertainment as long as different methods were used. Yet, it is hard to conceive the magnitude of these differences would be only due to differences in ascertainment methods. Genetic differences are not a likely explanation as childhood diabetes was associated with the same genotypes of the major histocompatibility complex in the Netherlands as in other north-western European countries (11). Over time, by comparing yearly incidence rates, remarkable changes in incidence rates have been noticed as well as two-fold differences between regions of the same country have been reported (1-5). However the numbers of newly diagnosed children involved in such breakdowns were small (10).

In north-western European countries the incidence of childhood diabetes (0-14 years) appears to have increased over the past decades (1, 5, 12). In the Netherlands the prevalence of diabetes among army conscripts at age 18 increased gradually from 0.99 per thousand in 1960 to 1.72 per thousand in 1980, indicating a similar trend. The incidence found in the present survey adds another suggestion: that of a possible north-south gradient over north-western Europe. This suggestion is by no means proven but reinforces the importance of exogenous factors involved in the clinical onset of childhood diabetes.

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§3. Discussion

The purpose of this discussion is two-fold:

1. to give an estimate of the prevalence from the incidence data and to compare these data to findings elsewhere;
2. to review data on viruses as exogenous factors in the etiology of childhood diabetes, as epidemiologic data have often been used as circumstantial evidence in this respect.

1. Prevalence estimated from incidence.

With certain provisos, one might attempt to estimate a prevalence from the incidence data, reprinted in the previous paragraph. If it is assumed the incidence of childhood diabetes did not change between 1959 and 1981 (the years in which the children encomprised in the incidence study were born) and if corrections for changes in mortality and migration are omitted, the calculated incidences per year cohort may be added. This summation represents cumulated yearly incidences rather than a true prevalence, and includes the abovementioned provisos. The results of this calculation (6) are given in the next table.

Table 1 Prevalence estimated from incidence rates, according to age group

Age in years	Annual incidence per 100.000 (1978-1980)	Estimated annual prevalence in 1980	
		n	per 1000
0- 4	6.8	142	0.2.
5- 9	11.0	598	0.6
10-14	14.4	1346	1.1
15-19	10.5	2149	1.7
0-14	11.1	2086	0.7
0-19	11.0	4235	1.0

An increase in the incidence?

The thus calculated annual prevalence of 18 year old boys for 1980 was 1.96 per thousand, the observed annual prevalence in army conscripts between 1978 and 1981 was 1.72 per thousand. The difference between this expected prevalence (projected from incidences observed 1978-1980) of 1.96 $^{0}/_{00}$ at age 18 and the observed prevalence at age 18 1978-1980 of 1.72 $^{0}/_{00}$ for the male sex represents an educated guess because of the provisos made. It would suggest that a further rise in the prevalence of childhood diabetes incidence might be expected during the next decade in the Netherlands. A rise in the incidence of childhood diabetes during the past decades was however found in other north-western European countries as indicated in the previous paragraph.

Comparison with other countries

Table 2A shows the magnitude of the differences in incidences found between 1970-1980 for children 0-14 years old in north-western European countries, performed on a nation-wide scale. Table 2B shows the methods used and the ascertainment-percentages.

Table 2[A] Studies of the incidence of diabetes mellitus in children up to 14 years old over north-western Europe (1970-1980).

Country	Population - seize	Time of study	Registration
Finland	1.03×10^6	1970-1979	hospital-files (retrospective)
Sweden	1.68×10^6	1977-1980	central registry (prospective)
Norway	0.95×10^6	1973-1977	hospital-files (retrospective)
Denmark	0.51×10^6	1970-1975	hospital-files (retrospective)
Netherlands	3.25×10^6	1978-1980	doctor's questionnaire (retrospective)

Table 2[B]. Ascertainment-procedures and incidence-rates found.

Country	Ascertainment	Percentage	Incidence per 100.000
Finland	drug-registry	94	28.6
Sweden	hospital-files	93	22.7
Norway	national insurance	91;93	17.6
Denmark	doctor's questionnaire death certificate army conscript data	88;98	14.0
Netherlands	patient's questionnaire	90	11.1 *

* after correction for ascertainment

To illustrate that the design of these epidemiological surveys may have profound influences on the findings: in Sweden (7) a retrospective study (1970-1975) was conducted (foregoing the prospective study in this country comprised in table 2A and 2B) in 7 different hospital areas with a total population of about 331.000 showing a mean yearly incidence of diabetes in children (0-14 years) of 19.6 per thousand. The prospective study (1977-1980) over the whole Sweden found an annual incidence of 22.7 per thousand. More importantly, differences were seen in the variables studied: notably there was a significant increase of boys under age 5 developing the disease compared to the former study (8). In both the retrospective and the prospective survey there was no seasonal variation in the onset of diabetes in children below age 5.

In the other studies of table 2 seasonal variation was also lacking in these very young children. In the dutch survey, reprinted

in paragraph 2, this association was absent until an age of onset of 10 years.

2. Viruses as exogenous factors

Peak-incidences in the autumn and winter months have often been taken to support the idea that viral infections might trigger the susceptibility to develop childhood diabetes. What is the evidence for this hypothesis from viral studies?

Several studies have proposed a relationship between insulin-dependent diabetes and Coxsackie virus (9), Rubella (10) and Mumps (12), next to Epstein-Barr virus, cytomegalia and poliomyelitis (11). The evidence for Coxsackie virus (type B) is strongest and will therefore be considered in some detail. A two-year prospective study in the Montreal area (13) found no differences in antibody titres to any of these single viruses in newly diagnosed diabetic children, compared to changes in antibody titres found in their healthy siblings. It is of note though, a fourfold rise in antibody-titre to one or more Coxsackie (B1, B3, B4, B5) viruses was found in 30 of the 109 diabetics studied as compared to 7 of the 72 healthy siblings. None of these diabetic children, as a subgroup, differed from the others, with regard to age or season of onset. This speaks against a common viral denominator as a single causative event of diabetes in childhood, despite the seemingly uniform clinical presentation of these children at diagnosis. Obviously, increases in viral antibody titres between 0 weeks (at diagnosis) and 4 weeks later, provide circumstantial evidence only for viruses as causative agents. An interesting observation has been that two Mumps-epidemics were associated with two peaks in the incidence of childhood diabetes with an interval of about three years (12).

Ji Won-Yoon et al. (9) reported direct evidence for viral involvement as the cause for a first diabetic ketoacidosis, implicating a variant of Coxsackie virus B4. They were able to isolate this virus from the pancreas of a 10 year old boy who died from this ketoacidosis, 7 days later. This, by itself, is a rare event in a clinic with much expertise in the treatment of that condition. It may therefore be that the sequence of events in this

particular patient was exceptional. Nevertheless, inoculation of mice with this human isolate produced hyperglycemia, inflammatory cells in the islets of Langerhans and beta-cell necrosis. In 1979 for the first time, these authors were able to implicate a virus as the cause of diabetes in a child, fullfilling Koch's four postulates. However, the best evidence that viruses have a causative role in insulin-dependent diabetes has come from animal studies. In mice, the M variant of encephalomyocarditis (EMC) virus can infect pancreatic beta-cells and produce a diabetes-like syndrome in certain inbred strains (14). In addition, variants of two viruses of human origin, reovirus type 3 and Coxsackie virus B4, could infect pancreatic beta-cells and produce a diabetes-like syndrome in, again, certain strains of mice (15,16).

How do these virologic data fit together? Concentrating on Coxsackie B4 for the moment, diabetes seems unlikely to be a common sequela of this infection, as antibodies to this virus occur in about half the population (17,18), whereas the prevalence of diabetes mellitus before age 19 is much lower. Antisera prepared to prototype strains of Coxsackie virus sometimes produce little, if any, neutralization of newly isolated strains (19). Differences in the degree of neutralization also occur among subpopulations of Coxsackie virus particles isolated from the same virus pool (20). Finally, continuous passage of Coxsackie viruses in animals as well as in cell culture can alter their antigenicity (19). Preliminary studies with monoclonal antibodies prepared against Coxsackie B4 indicate that this serotype does not represent a single virus, but many variants which differ antigenically (21). This implies that Coxsackie-viruses may at present be difficult to detect serologically as a cause of childhood diabetes, but this certainly does not exclude the possibility thereof. Epidemiologic evidence in England (22), the United States (14) and India (23), next to the prospective Montreal study as forementioned, support this view. Occasional reports of diabetes with rapid onset, during infections with Coxsackie B1 and B5 (24), B2 (25), B4 (26), B5 (28) in addition to the evidence provided by Ji Won-Yoon (9) generate a compelling issue.

Animal experiments supporting the evidence are reviewed briefly next.

In Ji Won-Yoon's case the isolated virus readily induced a diabetic condition in certain strains of mice, neutralization tests indicated a close relationship between the virus isolated from the childs pancreas and another strain of Coxsackie developed in the laboratory with the purpose of inducing diabetes in these mice. Strains of viruses generated to induce diabetes in laboratory animals are developed by enriching pancreatropic viral subpopulations through passaging the virus colonies on cultured beta-cells, obtained from those laboratory animals, mostly mice (29, 21). To such diabetogenic viral populations not all laboratory animals (mice) were susceptible. For example, the mouse-strains DBA/2J, SJL/J and Swiss were susceptible to such virus-populations, with the exception that DBA/2J were resistant to Coxsackie B4. BALB/c and C3H failed, in any case, to develop virus induced diabetes (15).

These experiments suggested the presence of a genetically controlled common receptor on the surface of beta-cells, shared by different viruses. In the case of encephalomyelitis and Coxsackie B4 virus induced diabetes, not all mice reacted within the susceptible inbred strains, although mice of the same age and sex were used. This suggests that intrinsic, non-genetic factors (e.g. metabolic factors) did have an effect on the extent of the virus induced diabetes. Successful attempts have been made to render mice more susceptible to virus-induced diabetes with treatments of subdiabetogenic doses of streptozotocin and glucocorticoids (32). The fact that glucocorticoids rendered these mice more susceptable to virus induced diabetes, may be related to the clinical history of patient 3 (Chapter I - paper 4).

Further evidence for non-genetic susceptibility to diabetes induced by streptozotocin comes from the observation that the likelihood of diabetes-induction is inhibited by oestrogens, but potentiated by androgens in BALB/C and C57BL/6 mice (31).

This is of interest in relation to the decline of the incidence of diabetes during puberty observed in girls, preceded by a peak at the onset of their puberty, whereas in males a gradual but

higher incidence was observed throughout puberty (paragraph 2).
Mice have been shown to develop insulin-autoantibodies after viral infections (34). This raises another pathogenetic possibility resulting in a modification of the presentation of diabetic syndromes, involving the hormone itself rather than the organ producing it. The question than must be raised whether viruses inducing diabetes in these laboratory animals are "pancreo-tropic" and/or "insulino-tropic". In the past 5 years we have noted the spontaneous occurrence of "classic" insulin-antibodies in 1 out of 59 (1978-1982) newly diagnosed diabetic children, i.e. prior to the administration of insulin. This occurred in 1978 in a four year old girl, but her sample was not further analyzed at the time, nor was it used for any of the studies of this thesis.
Another aspect of viral involvement in the genesis of experimental diabetes should be mentioned: some beta-cell cytotropic reoviruses can infect the anterior pituitary and produce hypophysitis (35). This finding may be related to the fact that 16 percent of newly diagnosed children had antibodies reacting with different cells of the hypophysis (36). The question whether viruses can be implicated in the cause of multiple endocrinopaties of which human diabetes mellitus takes part (adrenalitis, thyreoiditis, pernicious anemia, e.g. diabetes-type-1b - table 1, Chapter I), is at present unanswered.
Taken together, viruses may be pancreo-tropic, insulino-tropic and they may also be implicated in the autoimmunities towards other endocrine organs. Variants of known experimental viruses could cause this spectre of autoimmunities in suitable animals. Such variants may well go undetected by the serologic techniques used for the detection of the species the variants are derived from. Furthermore, it has been suggested the susceptibility of the infected animals to develop insulin dependency may be enhanced by sex-hormones and corticosteroids.
The difficulty to identify the nature of diabetogenic viruses remains, but again novel techniques may elucidate some of the problems. Dr. A.L. Notkins and his associates, at the National Institute for Dental Research in Bethesda, discussed some of their

latest results using RNA-fingerprints to identify variants of the EMC-virus (encephalomyelitis-virus) at the IVth International Workshop on immunology of diabetes, held in London in 1983 (37). They suggested that there are two variants of EMC; variant D which causes diabetes in some strains of mice and variant B which does not. Biologically they are very different, but serologically not. Only RNA fingerprints show the difference between these viruses. At least one spot, spot 7A, is present in the D variant, but not in the B variant. Mengo virus causes also diabetes in mice, but it rarely affects humans. It causes diabetes in animals much more rapidly than EMC virus. The difference between Mengo and EMC does not show up serologically, but there are obvious differences in the nucleotide sequence of these viruses. There may be two types of receptors in the cells of susceptible hosts, one for Mengo virus, one for EMC virus.

To investigate receptor susceptibility to infection they studied the binding of EMC virus and Mengo virus to cells. Each virus was internally labelled through radiation and added to a suspension of pancreatic cells in which the binding of the virus was measured. The rate of binding was rapid and (as expected) Mengo virus was found to bind faster than EMC virus. In other tissues of the body, such as the thymus and spleen, the binding rate was very slow. Other tissues were therefore considered negative and were not affected by the viruses (37).

These experiments show different affinities of viruses with a known chemical structure to specific organs e.g. the islets of Langerhans. The same group showed that the spleen cells of these mice could be stimulated with these viruses, indicating the viral receptors were in a dynamic state and could be modulated or induced. This provides strong evidence for heterogeneity in viral receptiveness. It may be that such receptor heterogeneity is associated with the clinical variability of the expression of islet-cell damage: children at toddler's ages seem to suffer from a more rapid devastation of beta-cell function of the pancreatic islets than older children (1).

The role of viruses in the etiology of childhood diabetes has further been underlined in the literature by the finding that one

third of the second cases in families already having a diabetic child would develop the disease clinically within three years of the first case (Chapter I - paragraph 5). In our own 22 multiplex families (Chapter I - paper 1) this was not the case. The time-span between the clinical onset of insulin-dependency between the first and the second child (before the age of 19 years) varied from 3 months to 13 years. Only 3 of 22 such sibpairs became insulin dependent within three years from each other. The development of the disease in parents, if present, was even further apart in time.

Such sequence of events questions the role of viruses in the sense of viral infections in man. What other pathogenetic mechanism apart from infection may be suspected from viruses in the etiology of type-1-diabetes?

Retroviruses are the only group of RNA viruses that replicate through a DNA intermediate. Some members produce tumors or non-neoplastic disease, others are not noticeably pathogenic. Retroviral genomes are essentially ubiquitous in vertebrate species and are maintained in nature predominantly as proviruses integrated in the host chromosomes, thus being transmissable to the progeny, as Mendelian genetic traits (endogenous retroviruses). The activation of viral genes does not result necessarily in the production of complete viral particles. Most often, the viral genome is only partially expressed (38). Products of endogenous retroviruses may stimulate specific humoral and cell-mediated immune responses in wild and laboratory animals. Natural antibodies to murine retroviruses are present in most mouse strains (39). Most recently, it has been demonstrated retrovirus genomes may be expressed at the pancreatic beta-cells of mice both in vitro and in vivo (38). It might be worthwhile investigating whether this tendency is associated with an immune response to retroviral antigens. As the expression of endogenous retroviruses is regulated by viral and host mechanisms and may be influenced by genetic and epigenetic factors, this hypothesis will be very difficult to test in man. Indirect evidence for this hypothesis may however be obtained by the finding of the integration of retroviruses to specific locations on chromosomes, in association with the ex-

pression of type-1-diabetes, analogous to the expression of some forms of leukemia and mouse mammary tumours by viral integration and activation of oncogenes.
Such mechanism of the involvement of virus particles would better fit the erratic and very partial inheritance of type-1-diabetes.
With this concept of genetic susceptibility and viral particles acting in concert to cause the disease (Chapter I), epidemiological surveys may "create an awareness of the nature of the environment in which disability arises, of the factors in the community which contribute to its causation and, in turn, its effect upon the community." The incidence in male toddler's may show other alterations over time than the incidence in, for instance, girls well into puberty. To monitor such alterations, ongoing prospective incidence studies are necessary. These prevail in Scandinavian countries. From Finland and Sweden a quite unexpected drop of 30 and 20 percent in the overall incidence of childhood diabetes (0-14 years), respectively, has been noted beginning in 1980 (Dr. J. Nerup, personal communication).
Another major advantage of prospective surveillance of new cases of childhood diabetes is that it would provide a structure for the detection of sibs at risk for the development of the disease from the moment the first family member developed the disease. Family members are the prime population for the consideration of immuno-preventive measures (Chapter I - paragraph 5).
With this perspective, the study reprinted in paragraph 2 might have served as a pilot-study for the feasibility of ongoing prospective studies in the dutch health-care system.
Should it not be possible to establish ascertained ongoing prospective surveys in the Netherlands, the presently identified relatively large group of diabetic children may be willing to cooperate with retrospective investigations into environmental circumstances. These circumstances might include the influence of the vaccination status (40), of parental age (41) and of nursing habits (42).

§4. Summary

The findings of Chapter II may be summarized as follows:

1. The incidence (newly diagnosed children per year) of childhood diabetes in the Netherlands under the age of 19 years is 10.9 per 100,000, under the age of 14 years 11.1 per 100,000.

2. There is indirect evidence for a rising prevalence with time in the Netherlands.

3. As in other surveys, young male children (0-4; 5-9 years) appear to be more susceptible than young female children, girls well into puberty have a lower incidence than pubertal boys.

4. A seasonal variation is not seen in the age-cohort from 0-9 years, for 10-19 year olds the incidence is higher in the autumn and winter than it is in the summer months.

5. No pronounced differences in incidence were found in the Netherlands between provinces nor with different degrees of urbanization of the living place at the time the first insulin injection was administered.

6. There appears to be a north-south gradient in the incidence of childhood diabetes over north-western Europe: the incidences reported are three times higher in Finland, two times higher in Norway and Sweden and slightly higher in Denmark, compared to the present rates in the Netherlands.

7. Viral studies in newly diagnosed children have only occasionally provided evidence for viruses as causative agents.

8. Classic serologic techniques to detect viruses as causative agents are likely to fall short to do so.

9. The mechanism by which viral particles induce insulin dependence in man may be examined by searching for integrations of retroviruses, known to be expressed on pancreatic beta-cells of mice.

10. For a better understanding of factors associated with the clinical expression of childhood diabetes ongoing, prospective, incidence studies are recommended.

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CHAPTER III TREATMENT

Primum nil nocere
(Galenus)

§1. An inventory of past and present trends

Table 1 is a schematic representation of factors involved in the treatment of any chronic disorder of childhood, defined as lifelong illness. Factors are arranged at three levels of interaction (A, B and C). Only those factors are included that underwent most change during the past ten years with regard to the treatment of childhood diabetes. The purpose of this arrangement into three levels of interaction is to demonstrate that until some ten years ago two levels of table 1 appeared to belong to a separate realm of the literature. Studies on "attitudes" (table 1 - A) were found almost exclusively in the psychosocial or public health literature. The instruments of the treatment proper (table 1 - C) were the domain of the classic medical journals. In the past ten years medical units - pediatric and adult - have started programs to study the effects of modes of therapeutic intervention at several levels of interaction. The determinants of therapeutic intervention (table 1 - C) are placed in the middle and are recurrent themes throughout the literature to date.

Table 1. A schematic representation of the dynamics of the strategy of therapeutic intervention in childhood diabetes.

A. Attitudes involved in therapeutic intervention.

The family	The child	The Therapist

B. Determinants of therapeutic intervention.

State of Disease	Aims	Location

C. Instruments of therapeutic intervention.

Practical means	Guidance	Education

Before that time, the scope of studies was usually limited to interactions within the levels indicated in table 1. For example, the need for patient-education has been recognized as long as insulin-substitution exists (1).
At pediatric ages the increased awareness of all three levels of table 1 interacting in the treatment of childhood diabetes came also from experiences with summer camps for diabetic children (2). As a consequence, hospital-units started adding social workers and psychologists to their teams mostly consisting of a nurse, a dietrician and a physician. With these facilities, the scene was set to study different levels of interaction in the same group of children, pioneered by Prof. Z. Laron in Israel, Prof. D. Etzweiler in the U.S.A. among other investigators.
It is of interest to note that countries, such as the Netherlands, that had no summer-camps for diabetic children, were late in establishing such teams. There was a well-known home for diabetic children with an experienced (para-)medical staff (Bosch en Duin 1972 - 1976). This however functioned as a residential care centre, despite efforts to have it function as an educational centre (3).
In this inventory of past and present trends the most significant changes in concept over the past ten years will now be mentioned or briefly reviewed:

A. Attitudes involved in therapeutic intervention.

1. The family.

The powerful role of the family in the management of childhood diabetes has been a recurrent theme throughout the literature. However, the studies used to be based on a linear model of family functioning. Interpersonal influences between parent (usually the mother) and child were seen as extending exclusively from parent to child. Because of its limits this approach has been seriously challenged (4). The focus of research has shifted to the broader family milieu, with an emphasis on patterns of cooperation and conflict among all members of the child's family in implementing

the diabetic regimen. Major factors affecting the familial impact were the time spent in the home managing the disease and the amount of change in family life-style that had to be made (5). The above-mentioned shift in research on family-interactions to all family members does not imply a denial of the mother as the person most intimately bound to the repercussions of the disease. Earlier studies indicated that the following 11 aspects of care were of concern to mothers of diabetic children: injections, diet, control, hypoglycemic episodes, urine testing, finances, amount of help and support available, time demands, independence of the child, feelings that the disease is a stigma and fears about the future (6,7).
A recent study explored how age, sex of the child, age at diagnosis and socio-economic status were related to the mother's perception of problematic aspects of care (8). It was found that, for example, the younger the child was at diagnosis, the more the mother worried about hypoglycemic reactions; a short duration of the disease was related to concern about insulin injections.
This recent study described interactions between the mother's concerns and states of the disease, whereas earlier studies explored mother's fears as such.

2. The child.

At all ages, the occurrence of diabetes super-imposes new tasks and coping demands. It poses significant challenges to the normal developmental processes of childhood. Actual consequences of this fact have not been drawn systematically however. This is hardly surprising as most health-care professionals, patients and families had no model that clarified the nature of such challenges in order to provide defined principles of effective treatment and preventive intervention.
Only recently multivariate models for coping with predictable crises have been developed. The model referred to (5) is based on a construct of crisis, coping and social support as well as on a developmental and life course perspective. The essence of this model is that a time of crisis is not only one of demand and dis-

tress, but also one of opportunity for a child that has most of its living time still to go. In predictable crises anticipatory coping can provide a basis for knowledgeable, competent health-care behaviour and for mastery and personal growth in the face of adversity.
Children show widely different levels of functional ability and personal satisfaction, independent of the degree of illness (9). Adopting a "wellness"- or a "sick"-stance is only very partially related to the state of the disease, as the perception of such stances during childhood will depend on family member interactions, as forementioned. The medical desirability of metabolic control in childhood is unlikely to become achieved unless the diabetic life is tolerated by the child as a routine in the background of otherwise normal development.

What are examples of predictable crises in childhood diabetes?
1. disease-specific emotionally distressing symptoms
   (nycturia, failure to grow, early chronic complications);
2. hospitalizations or increased frequency of hospital visits
   (fear for separation, impending separation, guilt-feelings);
3. response to minor acute complications
   (fear for injections, low bloodglucose without symptoms);
4. confrontation with therapeutic choices
   (seeing yet another specialist, advise of more insulin-injections per day);
5. failure to obtain a desired therapeutic response
   (high bloodglucose despite diet-keeping);
6. threat of coma
   (stepping up insulin-dosage, intercurrent illnesses);
7. jealousy or resentment by sibs
   (extra attention devoted to patient, having to share limitations of diseased sibling);
8. fear of loss of esteem by peers
   (not being invited on parties or with the engagement of romantic relations).

Readers familiar with the daily management of insulin dependency

will recognize each of such predictable crises and will be able to think of many more, challenging the emotional development of diabetic children.

3. The therapist.

In psychotherapy the attitude of the therapist is viewed as part of the medication. In somatic medicine there is the threshold of the power and magic physicians appear to operate with.
Even to date, systematic investigations into the characteristics of attitudes that are associated with positive patient-physician interaction, desired by patients, are relatively rare in the medical literature. Di Matteo et al (10) define the desired attitudes (not knowledge or ability) of physicians by patients suffering from chronic disorders as follows:

1. adopts a "person" rather than an "illness" orientation and is aware of developmental, psychological and socio-cultural factors;

2. establishes a relationship of trust, shows respect for family members and has a non-authoritan, responsive mode of communicating;

3. works out mutual agreement with the patient on treatment goals and takes both medical and psychosocial needs into account;

4. establishes a therapeutic team that includes the family, adjunct medical staff and physician as leader;

5. emphasizes patient education, self-motivation and personal responsibility for implementing the medical regimen;

6. is aware of predictable crises in the course of illness and provides anticipatory guidance, to minimize the impact and gives extra support during crises;

7. is aware of indications for counceling or psychiatric referral.

B. Determinants of the strategy of therapeutic intervention.

1. The state of the disease

A first requirement of knowledge on the nature and state of any disease is its frequency (Chapter II). Ascertained studies on the incidence of childhood diabetes in large populations are not older than ten years, with a few exceptions (11).
A second requirement of knowledge to the design of therapeutic strategy regards the "natural" course of childhood diabetes. This involves temporal courses - initial keto-acidosis, temporary remissions and the propensity of chronic complications - courses at different ages - toddlers - schoolchildren and adolescents - next to incidental courses - with infections, stress, excercise and with emotional upheaval - all of which go along with different requirements of insulin, hence strategies of therapeutic intervention. The delineation of these temporal, age-related and incidental courses of childhood diabetes has become increasingly more important throughout the medical literature of the past decades: the numbers of pages devoted to these aspects of the therapy of childhood diabetes in pediatric or diabetes textbooks has about doubled in the past ten years. The one characteristic of the disease that dominates all others with regard to the aims of treatment is the relation between the quality of the metabolic control of the treated disease and the propensity of a great variety of chronic complications. The past ten years have not resolved this issue. Conflicting editorials in reknown medical journals and numerous publications on the subject illustrated this. The recent initiative of the National Institutes of Health to reexamine this controversy by a multi-million dollar study is indicative of the persisting uncertainty. Both objectively quantifying degrees of vascular disease (12) and the accurate assessment of states of metabolic control (13) constitute major problems in resolving this classic dispute of internal medicine. In both fields signi-

ficant advances have been made. To mention two examples: elegant techniques to evaluate early states of retinopathy (14) and neurophysiologic studies on functional neuropathy (15). To illustrate the complexity of pathogenetic mechanisms underlying the controversy: it was recently suggested that glycosylation of collagen may result in the recognition of fibrous tissues as "foreign" by the immune-system (16).

Aims

Of course the aims of therapeutic intervention are in the centre of table 1. Although hotly disputed, they have changed little in the past ten years. Most pediatricians experienced in the treatment of childhood diabetes would agree with the following four aims:

1. normal physical development;
2. normal emotional development;
3. absence of overt acute symptoms of diabetes mellitus;
4. metabolic control as good as acceptable by the child.

Location

A marked change in the past ten years came with the introduction of methods for self-monitoring of bloodglucoses (17). Management of diabetes, unlike that of many other disorders, depends on active participation by the patient. This implies that diabetic control will be achieved by the patient at home and requires the establishment of a partnership between doctor and patient. Until the introduction of self-monitoring of bloodglucoses, it was customary to employ urine testing as an index of metabolic control. The limitations to the usefulness therof are presently widely accepted (17), excepting the use of urinary ketones to detect imminent metabolic disregulation at home (18).

C. Instruments of therapeutic intervention.

Education

The need for education of patients in treating their disease is as old as insulin itself and already Joslin indicated its paramount importance in 1924. In the past ten years separate sections of professional societies for diabetes education have started flourishing, in keeping with the notion that medical doctors are generally not trained to become teachers to their patients. Systematic studies evaluating various methods of teaching to patients or to patient groups have been published recently (19). Such studies comprised some quarter of the contents of diabetes journals as "Diabetes Care", established in 1976 through the initiative of the American Diabetes Association, to be followed by "Diabetic Medicine" in Europe in April 1984.

Guidance

Guidance is a terrible word as it pretends a lot while it may mean little by its passive significance. It is still used in the present context to indicate that scholarly knowledge and capability of patients to manage their diabetes with the means provided, does not imply patients will thereby do so. The weight of individual circumstances, prevailing in the homes of children with diabetes, cannot be overestimated. Adolescents may resent their diabetes to the point of consequent denial and mothers of diabetic toddler's may be so entrenched in their fear for nocturnal hypoglycemia, that it may become difficult to have them cut down on the number of fingerpricks to measure bloodglucose in their child. Individual circumstances require the development of adapted strategies for such and other sub-groups of diabetic minors. The development of such strategies has only recently begun, in particular for adolescents (20).

Means of treatment

Apart from the introduction of means for self-monitoring of bloodglucose, the most significant change of the past ten years was insight into the pharmaco-kinetics of various insulin preparations. The introduction of human insulins may represent progress to a lesser degree, as the immediate effectiveness thereof, compared to highly purified porcine insulins, differs hardly (21). It is now clear (22) that 60 percent of all variation in bloodglucose-profiles of treated patients is associated with variation in resorption from the subcutaneous depot, highly purified porcine- and human-insulin alike. It is perhaps surprising that it took more than half a century of insulin usage to arrive at this explanation. However, until recently, mono-iodinated insulin tracers suitable for the study of the subcutaneous fate of injected insulins, were not available. It should be noted that the above figure of 60 percent was derived under metabolic ward conditions, with standardized injection techniques. It may well be that 60 percent is an underestimate in every day life, especially in children resisting the injections, resulting in even larger variation in bloodglucoses.

Another new tool in the treatment deals with the evaluation of metabolic control: glycosylated hemoglobins. Although recently applauded in an Editorial of the New England of Medicine (13) its merits as an index of control in labile forms of childhood diabetes have been insufficiently assessed. In view of the great potential value of an independent measure of glycemic control in labile childhood diabetes, the clinical usefulness of different components of glycosylated hemoglobins was investigated in the second paper reprinted in this chapter.

The first paper reprinted is a comparative study on modes of health-care delivery to diabetic children. Many of the factors of the strategy of therapeutic intervention aforementioned were involved in this comparison. Present trends in therapeutic intervention were incorporated, that were either emerging or ongoing at the moment the home-care study was initiated. It should be emphasized this was possible as all participants of the team for

the home-care had extensive experience with the treatment of childhood diabetes before. An important aspect of this study, its experimental design, is reported on first in the next paragraph.

§2. Design of the home care program

The aim of the home-care-study, reprinted in the following paragraph, was to investigate whether "home-care" might be a better and cheaper treatment modality for childhood diabetes than the current "hospital-based" systems in the Netherlands.
The terms "home-care" and "hospital-based" suggest that the location is the prime difference between these two systems.
As will be demonstrated in the reprint, also the amount and type of education offered to the families differed, next to the availability of materials for the self-measurement of bloodglucoses at home, of a 24 hours telephone service and of regular advice by child-psychiatrists to the team treating the children.
The keyperson in the execution of the home-care-program was and is the nurse practioner visiting the homes of the children.
First, a prospective random assignment of all children attending the diabetes clinic to either home-care or continuation of hospital-based-care was considered. In the original proposal to the Ethical Committee of the Sophia Children's Hospital early in 1978, a comparison between the results obtained in 18 months time in each group was planned. One of the provisos made by the Ethical Committee, worded by Dr. E. BenGershôm, was that this approach needed to be interrupted, as soon as it became obvious that children with home-care were better of in terms of admission-rates for metabolic disregulation or emergency visits to the out-patient department.
The nurse practioner engaged in this study, Sister J.J. de Visser, started her work in September 1978. Her excellent training and subsequent experience in the ongoing treatment of children with diabetes from her previous position as nursing director of "Bosch en Duin" (home for diabetic children - Dr. S.G.Th. Hulst, medical director) soon paid off: in three months time, before Christmas 1978, the abovementioned proviso of the Ethical Committee appeared prophetic. Six children assigned to the "home-care-group", known with a variety of problems with regard to their diabetes, improved considerably. It was obvious that children suffering from similar problems, but assigned to the other study-

group, could morally not be withheld from the home-care for one and half a year for scientific purposes only.
The randomized prospective design of the investigation comparing "hospital-based" versus "home-care" was then changed into a longitudinal follow-up comparing relevant parameters before home-care was introduced (September 1977-September 1978) to changes found in a three year follow-up (September 1978 - September 1981) offering all children attending the clinic home-care at the same time.

§3. Addendum

Paper 1

Home Care for children with diabetes mellitus in the Netherlands

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Introduction

A three-year longitudinal study was done to compare effects of (traditional) "hospital-based-care" versus "home-care" (table I). As parameters of effect three variables were chosen: (1) Admission rates to the hospital for newly diagnosed diabetes and pre-existing diabetes, because of metabolic disregulation. (2) "Acute" (i.e. non-planned) outpatient clinic visits, because of metabolic disregulation. (3) The quality of metabolic control as evidenced by glycosylated hemoglobin.

The crucial role of the nurse-practioner can be appreciated from her 5-fold task: (a) To evaluate knowledge and ability of diabetic self-management as taught in five evening sessions, concluded by an examination. (b) To evaluate the execution of clinical advice at home and modulate it in timing and to individual family circumstances. (c) To visit homes in case of threatened disregulation. (d) To ensure medical and social adjustment at school (half of the children gave a talk in the classroom. "Is diabetes contagious?" was a common question). (e) To signal long-term psychosocial complications. These were discussed by the diabetes team including a child psychiatrist once monthly.

Table I. Sophia Children's Hospital - Diabetes Clinic

	A Hospital-based care	B Home care
Team	2 pediatricians 3 dieticians (no nurse-practitioner)	2 pediatricians (the same 3 dieticians as in A) 1 nurse-practitioner
Outpatient visits	3 - 4 times per year	3 - 4 times per year
Telephone services	to pediatric resident-on-call only in emergencies	to nurse-practitioner or pediatrician, 24 hours a day, encouraged
Metabolic control	fractionated 24-hour urines collected at home every 3 weeks on Sundays	glycosylated hemoglobins at each clinical visit self-monitoring by urinary and blood strips
Patient education (classes)	1 evening a year on mixed topics no structured program	5 evenings a year structured program directed at self-care
Patient education (individually)	at clinic visits by dietician and doctor 2 - 3 times a year	at home (5 - 15 1-hour visits to each patient) at clinic visits by dietician, nurse and doctor, 3 - 4 times a year
Child-psychiatry	only in emergencies	round-table meeting with diabetes-team-members every 4-8 weeks, 1 hour

Subjects and Methods

Table II shows the design of the study and characteristics of the 41 children out of 78 selected for the longitudinal study comparing the (traditional) hospital-based system and the home-care system. All children who had diabetes for 1.5 years or longer by September 1978, using 0.6 units of insulin or more per kilogram body weight per day, which excludes children with protracted remission phases, were included. Newly diagnosed patients were all excluded for the longitudinal comparison, as well as referrals of children with treated diabetes. From September 1977 to September 1981, 11 patients were referred to internists because of their age (15.6 - 18.1 years). Their average HbA_1 was 12.1%.
Temperature-controlled "Isolab" minicolumns were used to measure HbA_1. Bloods were drawn on ice at each clinic visit. Samples from patients to serve as internal standards were chosen, according to suspect quality of control, yielding high, medium and low diabetic HbA_1 percentages. Standards were stored for 2 - 12 months and measured every week. The results are expressed as means ± 1 SE.

High HbA_1	($>$ 13%)	: 18.0 ± 0.4%	n = 10
Medium HbA_1	(10 - 13%)	: 12.3 ± 0.3%	n = 9
Low HbA_1	($<$ 10%)	: 7.4 ± 0.2%	n = 8

This implies an average coefficient of variation between assays, using the micro-column (Isolab) system, of 10 - 15% or, in terms of percentage $HbA_1 \bar{x}$ ± 1.0% (95% confidence limits).*

* Total-glycosylated hemoglobins were assayed, e.g. without discarding the fast-glycosylating component (paper 2).

Table II. Sophia Children's Hospital - Diabetes Clinic

	Sept. 1977 to Sept. 1978	Sept. 1978 to Sept. 1979	Sept. 1979 to Sept. 1980	Sept. 1980 to Sept. 1981
Number of children	41	41	41	41
Average age	9.9	10.9	11.9	12.9
Age range, years	4.0-13.8	5.0-14.8	6.0-15.8	7.0-16.8
Mean diabetes duration, years	5.3	6.3	7.3	8.3
Duration range, years	1.6-9.9	2.6-10.9	3.6-11.9	4.6-12.9
Mean HbA_1, %	-[1]	12.9	13.2	12.7
Range HbA_1, %	-[1]	10.2-19.3	9.2-18.1	9.1-18.7
	hospital based	home care		

[1] Not done

Table III. Hospital admissions at Sophia Children's Hospital Diabetes Clinic

	Sept. 1977 to Sept. 1978	Sept. 1978 to Sept. 1979	Sept. 1979 to Sept. 1980	Sept. 1980 to Sept. 1981	Difference factor 1977-1980
Number of children seen	78	88	99	106	
Admissions days	160 (2.4)*	65 (0.8)*	38 (0.4)*	8 (0.08)*	- 30x
Metabolic disregulation (excluding newly diagnosed) "acute" visits	24 (0.3)*	10 (0.09)*	3 (0.02)*	1 (0.01)*	- 30x
Newly diagnosed	11	10	12	8	
Admission days	209 (19)	141 (14)	40 (3.3)	26 (3.2)	- 6x
	hospital based	home care			

* the numbers between parentheses represent averages per child in the years indicated

Results

Table III lists the hospital admission rates before and during the home-care system. Also the number of "acute" (i.e. non-planned) clinic visits are shown. The numbers between parentheses indicate the number of hospital days and acute visits per child. Finally the numbers of hospital days for newly diagnosed children (initial treatment) are given. With the home care the number of hospital days, because of metabolic disregulation, excluding initial treatments, decreased 30 times, as was the decrease in acute visits.

The first HbA_1 value during the period September 1978 to September 1979 in the 41 children was 14.2% compared to an average value of 12.9% for the whole year, suggesting an improvement associated with the change in medical regimen. Girls in puberty did much worse than boys, their average HbA_1 over the 3 years followed was 14.3 versus 12.2% in boys. Whereas boys were able to maintain the same level of control before and in puberty, the pubertal girls did not.

Discussion

As early as 1924 Joslin (1) recognized the paramount importance of diabetes education to adults and children alike. Since diabetic regimens involve diet, insulin injections, testing of urine or blood, diabetes treatment became teamwork, including dieticians, nurses and doctors (2). Centres treating childhood diabetes have added social workers and psychologists or child psychiatrists to these teams, as it became obvious that psychological stability of the families of these children was a major determinant for the outcome in terms of hospitalization and quality of metabolic control (3).

Approaches such as these have indeed led to fewer hospitalizations and better metabolic control, as evidenced by patient's urinary test results (4). From this achievement on, pediatric centres have focused on behavioral or psychologic issues, such as coping styles (5), compliance (6), maternal emotional adjustment

(7). More recently the focus of this sort of research has shifted to the broader family milieu (8) and it became apparent that all family members are active and interdependent contributors to the effectiveness of diabetes management at home. If one accepts this viewpoint it is obvious that the home of the children deserves more attention. The 96% acceptance rate of the families receiving the nurse-practioner in their homes 2 - 15 times per year illustrates that she was not felt to be an intruder into family intimacies nor to impose unintelligible or impossible medical desirabilities on the families.
In a country with small distances and with an unbridgeable gap between the hospital and the homes, as all internists and pediatricians act as consultants, the visiting experienced nurse-practioner is a crucial and economic adjunct to the care for diabetic children.

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Paper 2

Limitations to the use of glycosylated hemoglobins as a parameter of glycemic control in childhood diabetes

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Abbreviations used:

HbA_1 (%) = glycosylated hemoglobins, percent of total hemoglobin

$t\text{-}HbA_1$ (%) = total-glycosylated hemoglobins, percent of total hemoglobin

$f\text{-}HbA_1$ (%) = fast-glycosylated hemoglobins, percent of total hemoglobin

$s\text{-}HbA_1$ (%) = stable-glycosylated hemoglobins, percent of total hemoglobin

IEF = iso-electric-focussing

bgl = bloodglucose concentration in mMol/L.

Summary

The clinical utility of glycosylated hemoglobins to interprete degrees of hyperglycemia in childhood diabetes was investigated by *in vitro* and *in vivo* studies. Two components of glycosylated hemoglobins (fast- and slow-glycosylating) were measured by standardized micro-column-cationexchange chromatography. Stable-glycosylated hemoglobin assayed by this method correlated well with iso-electric-focussing ($r = 0.92$).

Red-cells of normal subjects and diabetic children were incubated with physiologic and supraphysiologic (25 mMol/L) glucose to stu-

dy the formation of fast-glycosylating hemoglobin after 24 hours incubation. Red-cells were separated according to their age in the circulation by discontinuous density gradients.
Young red-cells already contained 60 percent or more of the glycosylated hemoglobins of older or all red-cells, both in normals and in diabetic children. These young red-cells acquired fast-glycosylated hemoglobins much more readily on incubation with 25 mMol/L glucose for 24 hours than older red-cells.
Glycosylated hemoglobins in young red-cells, fast-glycosylated hemoglobins and stable-glycosylated hemoglobins in all red-cells were compared to different time-courses of glycemia in eight labile diabetic children over eight weeks time. The findings caution against the use of these measures of glycosylated hemoglobins as parameters of hyperglycemia in labile type-1-diabetic children: correlations between any of the measures and time-courses of hyperglycemia never exceeded $r = 0.6$ by Spearman's test.
The determination of t-HbA_1, f-HbA_1 and HbA_1 content of erythrocyte-age-separations did not reflect the glycemia of labile diabetic children.

Introduction

To obtain an index of previous mean glucose concentrations over weeks or months, the measurement of glycosylated hemoglobins (HbA_1) is advocated (1,2). HbA_1 are known to consist of at least two components, one fast-glycosylated (f-HbA_1), the other stable-glycosylated (s-HbA_1) (3).
In this investigation the significance of these two measures to retrospectively interprete time-courses with different degrees of hyperglycemia in labile childhood diabetes was explored.
First, the most commonly used method for the assay of HbA_1 (micro-column-cationexchange chromatography) was compared to isoelectric-focussing (IEF). Given a satisfactory comparison, a standardized micro-column-procedure was used throughout the study.

Second, the time kinetics of the formation of f-HbA_1 and s-HbA_1 from glucose was studied in vitro. This was done before and after separating the red-cells according to age on discontinuous density gradients and subsequently incubate these red-cell populations with normal and supraphysiologic glucose concentrations. These procedures provided data on the content and saturability of total-HbA_1 (t-HbA_1), f-HbA_1 and s-HbA_1 in young and older red-cell populations in the circulation of diabetic children, compared to those of normal subjects.
These measures were then compared to blood-glucose-profiles found over eight weeks time in eight labile diabetic children staying in a residence.

Methods

All assays took place within three hours after sampling. Heparinized blood aliquots were kept at 4°C in the meantime.

1. Columnchromatography for HbA_1

Micro-columns (Isolab^R Akron, Ohio, U.S.A.) were used according to the procedure from the manufacturer and kept in a constant temperature-water-bath (22°C), while standard hemolysates were co-measured in all tests. These standards of t-HbA_1 (diabetic plus non diabetic) were kept at -70°C and results of these were as follows (means ± S.E.)

High HbA_1 standard	(>13%)	: 18.0 ± 0.4%	n = 10
Medium HbA_1 standard	(10 - 13%)	: 12.3 ± 0.3%	n = 9
Low HbA_1 standard	(<10%)	: 7.4 ± 0.2%	n = 8

This implies an average coefficient of variation between assays, using the micro-column-(Isolab)-system, of 10 - 15% or, in terms of percentage HbA_1: $\tilde{x}$ ± 1.0% (95% confidence limits).

To determine s-HbA_1 blood was washed two times, the erythrocytes diluted in 1:10 saline and incubated at 37°C for six hours. To determine t-HbA_1, 50 microliter of whole blood was immediately hemolysed.

2. Iso-electric-focussing (I.E.F.)

This method was originally described and modified by Jeppson (4). LKB Ampholine^{R} PAG-plates (LKB-Produkter AB, Sweden) were used. The coefficient of correlation (Spearman's test) gave a rho of 0.92 for IEF versus s-HbA_1% by micro-columns chromatography for 45 samples over a s-HbA_1 range of 7 - 16 percent.

3. Incubations of erythrocytes

Blood was taken by venapuncture from six diabetic and four non-diabetic subjects. Red-cells were washed three times in saline. t-HbA_1 was determined before the red-cells were incubated for six hours in saline to determine s-HbA_1. After this the cells were washed and diluted to a hematocrite (Ht) of 0.50. A sample was then taken to determine s-HbA_1.

Incubations were in basal medium Eagle (BME) with Hanks salts (Gibco^{R} biocult) with added adenine, bicarbonate and glucose to physiologic concentrations. A 37°C incubator with 5 percent CO_2 was used. Cells were suspended in the medium and put in Petri-dishes. Dilution was at an hematocrite of 0.20 l/l, glucose concentrations used for incubation were 5 and 25 mMol/l. HbA_1 was determined every four hours until a maximum of 24 hours. After harvesting the erythrocytes, a sample of the medium was taken to exclude hemolysis. The cells were then washed three times and glycosylation measured as f-HbA_1 and s-HbA_1. Young cells and unfractionated erythrocytes (see below) were incubated, one in 5mMol/l glucose, the other in 25 mMol/l glucose. Diabetic as well as non-diabetic erythrocytes in sterile conditions were studied.

4. Density separation of erythrocytes according to age

A modification of the method described by Rennie et al (5) was used: discontinuous gradients were prepared to harvest a greater and quantifiable amount of erythrocytes of a different age. This was done by taking different mixtures of BSA-Percoll^{R} (Pharmacia, Sweden) and the BSA-watér solutions and carefully layering these on top of each other by means of threechannel-peristaltic pumps. First testruns were made with 74 - 85 percent BSA-Percoll^{R} with steps of 2 percent. For each layer 1.5 ml was used. After the

testruns a suitable gradient was chosen with narrower ranges of percent BSA-Percoll^{R}, to yield five different layers (red-cell age groups). Ten tubes were used for each person.
After washing in saline, 1.5 ml of suspended erythrocytes (Ht = 50 percent) were layered on the top of these gradients. Centrifugation was according to Rennie et al (5).
A small amount of clumped cells was exclusively found in the bottom layer and data from this layer were excluded from further analyses. The method separated young cells from old cells: no reticulocytes were found in layers lower than the top one. For cell-age control various tests were used immediately after separation. 2,3-diphosphoglycerate (2,3-DPG, mMol/l ery) (6), glucose-6-phosphate dehydrogenase (G-6-PD, mU/10^{9} ery's) (7), pyruvatekinase (PK, mU/ 10^{9} ery's) (7), reticulocytes ($^{0}/00$) and mean cellular volume (MCV, fl) (6) were decreased in older erythrocytefractions, as expected. These parameters were determined in young (layer 1), medium (layer 3) and old erythrocytes (layer 4). Hematological indices were calculated after determination of Hb, Ht and erythrocytes number in a Coulter counter. Enzymes and 2,3-DPG were tested with Boehringer^{R} Testkits (Boehringer-Mannheim, FRG). All determinations were in duplo. The results are in table 1.

Table 1. Density red-cell separation of blood of four diabetics and four normal subjects: data expressed (± 1 S.D.) as percentage of all washed cells (100%) of diabetics (upper half) and of normals (lower half)

	reticulo-cytes	MCV	HbA_1	G-6PD	PK	2,3-DPG
washed cells	100%	100%	100%	100%	100%	100%
young cells (layer 1) Diabetic	266±53	104±3.0	82±4.0	244±114	223±45	140±20
old cells (layer 4) Diabetic	0%	98±1.5	106±3.5	97±20	70±21	80±32
young cells (layer 1) Non-Diab.	250±20	101±0.4	85±3.8	198±12	131±12	110±10
old cells (layer 4) Non-Diab.	0%	98±2.0	109±5.0	86±11	57±21	45±12

Patients

An 8 weeks' study with eight diabetic children was conducted at the Kinabu medical residence (consultant pediatrician A.v.R.), to which the children were admitted because of inadequate metabolic control, with or without psychosocial problems. The eight children (mean age 13.6 ±2.2 years, range: 10-16) and their parents gave their written consent to have bloodglucose-determinations by fingerprick, urinary glucose and ketones twice daily in addition to 24 hours-bloodglucose-profiles once a week, during 8 weeks. This "home"-monitoring was under day and night supervision of the health-care-personnel as was the administration of insulin. Bodyweight was determined once a week. Changes in diet and insulin were done by one of us (A.v.R.), who was kept unaware of the HbA_1 results. Four children and parents also agreed to have venapunctures once a week to determine additional HbA_1 measures, including erythrocyte-age-separation. Daily records of each child were kept of daily activities, symptomatic hypo/hyperglycemias and intercurrent illnesses.
All children were well beyond the remission-phase of their diabetes and were on combinations of long and short acting highly purified porcine insulins twice daily. The findings on HbA_1% and on insulin dosages and diets used are in table 2.

Table 2. Total and stable glycosylated hemoglobins (± 1SD) in 8 labile diabetic children over 8 weeks time with averaged insulin dosages and caloric intake.

patient	mean t-HbA_1	mean s-HbA_1	change in s-HbA_1 week 1 - 8	mean insulin/U/kg/day	mean Kcal/kg/day
1.	14.4 ± 0.8	12.9 ± 0.7	+ 1.1	0.8	25
2.	12.8 ± 0.8	11.9 ± 0.6	- 0.1	0.4	55
3.	15.7 ± 0.5	13.9 ± 0.6	- 0.5	0.6	28
4.	11.6 ± 0.8	10.4 ± 0.4	+ 0.7	0.7	41
5.	12.9 ± 0.7	12.1 ± 0.8	- 0.4	0.6	52
6.	14.9 ± 0.6	13.8 ± 0.8	+ 1.9	0.8	39
7.	12.3 ± 0.8	11.1 ± 0.5	- 0.4	1.2	49
8.	10.8 ± 2.1	9.4 ± 0.2	+ 0.7	0.9	68
means	13.2 ± 1.7	11.9 ± 1.6			

Changes in s-HbA_1 (95 percent confidence limits - 1% s-HbA_1) between the beginning of the study (week 0) and the end (week 8). There was no correlation ($p < 0.05$) between the averaged insulin dosage and the averaged calories taken per day. Patients 1 and 3 used most insulin compared to their caloric intake.

Bloodglucose and urine analysis

Bloodglucose-determinations were done with Ames-glucometers^R (Ames Netherlands B.V., Mijdrecht) which correlated well capillairy GOD-determinations ($r = 0.93$).

M-values were calculated as the index of glycemic excursions for the whole period according to Schlichtkrull et al. (8). All children at the residence had M-values above 30, confirming the lability of their diabetes.

Urine analysis was done with Ames-Glucosticks^R and -Ketosticks^R twice daily.

The findings on bloodglucose-profiles and M-values are in table 3.

Table 3. Glucose-profiles in 8 labile diabetic children over 8 weeks time.

patient	age in years	mean blood-glucose in mMol/L	mean bgl. during first 2 weeks minus mean bgl. during last 2 weeks	mean fasting bgl.	mean of 24-hours profiles of bgl.	mean M-value (see text)
1.	16	16.5 ± 2.4	+ 2.5	15.4 ± 2.3	15.7 ± 3.0	68
2.	12	10.5 ± 3.7	+ 4.2	9.9 ± 3.1	10.5 ± 3.7	32
3.	15	10.2 ± 6.9	- 6.4	9.5 ± 6.3	9.9 ± 4.5	67
4.	16	11.0 ± 5.5	+ 1.0	10.0 ± 3.9	9.2 ± 3.8	50
5.	10	11.7 ± 4.3	- 0.6	10.0 ± 3.9	12.1 ± 4.4	45
6.	12	12.4 ± 5.8	+ 6.2	9.9 ± 4.8	11.3 ± 2.8	61
7.	15	9.6 ± 3.8	+ 0.3	8.3 ± 1.7	11.3 ± 2.3	33
8.	11	12.4 ± 5.0	+ 0.5	12.1 ± 4.9	11.3 ± 2.8	55
means:	12.4	11.8 ± 2.2		10.8 ± 2.2	11.4 ± 2.0	51

Patients 1 and 3 were selected for a visual comparison between bloodglucoses and glycosylating components of HbA_1 (figure 2).

Results

1. In vitro studies: incubation of red-cells of diabetic children and normal subjects.

Table 4A contains the findings by incubating red-cells at 5 mMol/L glucose (normal concentration) and 25 mMol/L glucose (supraphysiologic concentration) in young and all red-cells of four normal subjects. Table 4B contains the same data on red-cells obtained from six diabetic children.

By these short-term incubations the s-HbA_1 content did not change and the difference between "starting s-HbA_1" and "final t-HbA_1" was due only to increment of f-HbA_1, as indicated next

in figure 4A. Young red-cells acquired f-HbA_1 significantly faster on incubation in 25 mMol/l glucose, both from diabetics and from normals. Calculated slopes in increment were respectively 0.20 and 0.17 for these categories, whereas all red-cells had slopes of 0.06 and 0.09 for diabetics and for normals. The findings demonstrate different kinetics for the formation of f-HbA_1 in young cells as a fraction of all red-cells.
Furthermore, young red-cells before incubation contained already 60% or more of the HbA_1 contents of all red-cells. This relationship was also found in 30 other diabetic samples, not comprised in figures 4A and 4B. In these 30 samples the average f-HbA_1 content (t-HbA_1 - s-HbA_1) was 1.7% HbA_1 with a range of 0.5-3.0%.

Table 4A. Increment in f-HbA_1 by the incubation of red-cells in two glucose-concentration over 24 hours in young red-cells and all red-cells of four normal subjects - means and ranges.

	Starting s-HbA_1%	Final t-HbA_1%	Calculated slope of increment
All red-cells			
in 5 mMol/L	7.6 (6.5-8.1)	7.3 (5.5.-9.1)	0
in 25 mMol/L	7.6	9.1 (8.6-9.5)	0.09
Young red-cells			
in 5 mMol/L	5.6 (4.9-6.3)	7.9 (7.5-8.3)	0.12
in 25 mMol/L	5.6	8.9 (8.6-9.2)	0.20

Table 4B. The same experiment as in table 4A, for red-cells of six diabetic children.

	Starting s-HbA_1%	Final t-HbA_1%	Calculated slope of increment
All red-cells			
in 5 mMol/L	13.8 (13.5-14.7)	14.0 (13.3-14.9)	0.01
in 25 mMol/L	13.8	15.4 (13.3-16.7)	0.06
Young red-cells			
in 5 mMol/L	- not done -		
in 25 mMol/L	7.7 (6.7-8.9)	11.1 (10.7-12.1)	0.17

2. Clinical studies: Comparison between bloodglucose-profiles and HbA_1

All measures of glycosylated hemoglobins (f-HbA_1, t-HbA_1, s-HbA_1 of all red-cells and t-HbA_1 of young red-cells) were compared to glucose-profiles of the eight children studied over 8 weeks (glucose in the bloodsample itself, 24 hours bloodglucoses on the previous day, the same day, the previous 1 - 8 weeks and all 8 weeks). Glucosuria was also compared to these measures of HbA_1. The linear regression coefficient was never greater than 0.6 excepting for a comparison between:

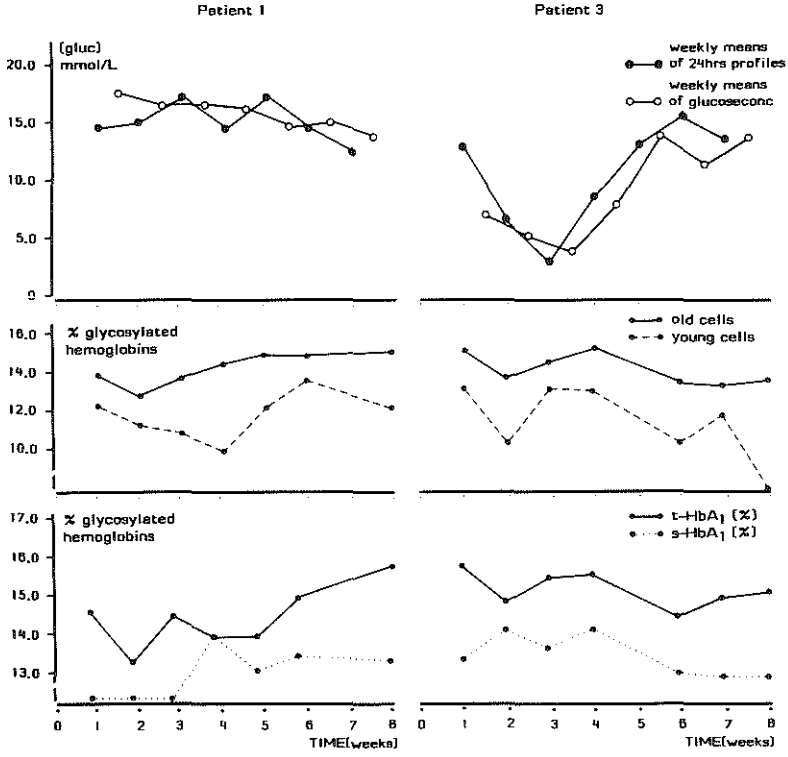
- the bloodglucose of the sample itself and t-HbA_1 ($r = 0.96$) and s-HbA_1 ($r = 0.91$);
- mean of 24 hours bloodglucoses the same day and t-HbA_1 ($r = 0.60$)

It was to be expected that the mean of these widely fluctuating bloodglucoses over the 8 week period correlated only weakly to final glycosylated Hb-values (t-HbA_1: $r = 0.34$, s-HbA_1: $r = 0.20$).

To illustrate the lack of correlation between components of

HbA_1 and episodes of glycemia in these labile patients visually, the clinical time-course of two children is represented in figure 2.

Figure 2.


Patient 1
Patient 3
(gluc)
mmol/L
20.0
15.0
10.0
5.0
0
weekly means
of 24hrs profiles
weekly means
of glucoseconc
% glycosylated
hemoglobins
18.0
14.0
12.0
10.0
old cells
young cells
% glycosylated
hemoglobins
17.0
16.0
15.0
14.0
13.0
t-HbA1 (%)
s-HbA1 (%)
0 1 2 3 4 5 6 7 8
TIME(weeks)
0 1 2 3 4 5 6 7 8
TIME(weeks)

In the upper panel averaged bloodglucoses of 24 hours profiles (closed circles) and means of daily bloodglucoses (open circles) were plotted against time. In patient 1 high, but stable bloodglucoses were seen, whereas patient 3 had (symptomatic) hypoglycemia during week 2 and 3, despite the temporary decrease of her insulin dosages.
In the middle the t-HbA_1% of both young and old cells failed to show a significant decrease up to five weeks after this hypoglycemic episode in patient 3, whereas patient 1 showed a decrease in t-HbA_1% of young cells during the first three weeks, not reflected by the expected closely time-related changes in bloodglucoses.

Discussion

Although the existence of fast- and stable- glycosylating components of HbA_1 has been well recognized (9,10,11), the time-kinetics of the formation of these two components have not been resolved (10). Yet the separate measurement of these two components might be useful if fast-glycosylation reflected glycemic control over shorter periods and stable-glycosylation over longer periods of time.
In the present study fast-glycosylation was examined by the separate assay of f-HbA_1 (t-HbA_1 minus s-HbA_1) and by the HbA_1 contents of young erythrocytes, that have had shorter exposure times to the glucose of the plasma than older red-cells.
The precision of the micro-column system used was 1 percent of HbA_1 (95 percent confidence limits), s-HbA_1 thus measured compared well to the IEF-method.
In individual samples f-HbA_1 did not correlate with either t-HbA_1 or s-HbA_1, endorsing the premise that f-HbA_1 might separately reflect short episodes of hyperglycemia. This however could not be demonstrated from the correlation with glycemia over short periods of time in eight labile diabetic children.
Also, the HbA_1 content of young erythrocytes failed to do so, which could be explained by the finding that these young red-cells contained already 60 percent or more of the total HbA_1 con-

tent of all red-cells, despite their shorter living time in the circulation. Furthermore, young red-cells (of normals as well as of diabetic children) acquired HbA_1 faster than all red-cells on incubation in supraphysiologic glucose-concentrations.

These findings suggest that shorter episodes of glycemia cannot be detected by either f-HbA_1 or by the HbA_1 content of young red-cells. The red-cell separation method was validated by the finding of the expected differences in hematologic as well as biochemical parameters for red-cell age. Red-cells from layer 4 in the present discontinuous density separation experiments correlated with age parameters for all red-cells, including HbA_1, indicating this separate represented average red-cells. This is explicable by the fact that the young cells thus separated comprised only 10 percent of the red-cells (6,10). In keeping with this study Spicer et al (12) found that incubation of red-cells with different glucose-concentrations showed a linear increment of the HbA_1-content with time, in red-cells of normals and of diabetics. As in this study, this increment was due to f-HbA_1 and not to s-HbA_1. Gillery et al (13) reported that young cells are capable of actively transferring glucose to hemoglobin by means of a red-cell membrane fraction. Kinetics might therefore differ in young cells. Differences in passive glucose permeability between red-cells were found by Higgins et al (14). Furthermore glycosylated products with other time-kinetics may be assayed by the present methods as recently suggested by Garlick et al (15). These authors found that by rechomatographing "HbA_{1c}" (from Biorex-70 columns) on a Glycogel B boronate affinity resin gave a 70 percent adherence indicating that less than 30 percent of the "HbA_{1c}" consisted of hemoglobin with beta-chain terminal valine-bound glucose. The remainder of this "HbA_{1c}" was glycosylated HbA_0, with 14 percent alpha-chain N-terminal valine-bound glucose, 40 percent alpha-chain lysine- and 46 percent beta-chain lysine-bound glucose. The kinetics of these compounds are different, compared to each other. This may be connected to the present finding, that even s-HbA_1 correlated with glucose of the sample itself.

To circumvene the co-chromatography of glucose-adjuncts to hemo-

globin other than at the beta-chain terminal valine (HbA_{1c}), the thiobarbituric-acid-method has been propagated. One of the problems with this method however is that the colour-development differs with the incubation conditions of the hemolysate and with the use of acetic acid instead of oxalic acid in the incubation mixture (16).
In this study there was no relation between the formation of components of HbA_1 and the glycemic control in labile diabetes of childhood. The determination of fast glycosylated products or the HbA_1 content of young erythrocytes did not reflect shorter episodes of glycemia, studied over 8 weeks time.

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§4. Additional findings with home care

The purpose of this paragraph is to communicate additional findings with the home-care project. These findings relate to:

a. Patient-selection.
b. Nature of admissions and acute visits.
c. Patient education.
d. Self-monitoring.
e. Metabolic control.
f. Economy of home-care.

a. Patient-selection.

The deviation from a randomized prospective design with the home-care study (described in paragraph 2 of this chapter) necessitates a more detailed account of the selection of patients that participated in the comparison between "hospital-based" and "home-care". This comparison dealt also with HbA_1% before and after the introduction of the home-care system (table II of paper 1 - page 198) and involved 41 children.
In the year before the home-care system was introduced 78 children (table III of paper 1 - page 199) visited the diabetes clinic, from which 78 - 41 = 37 were excluded by the following criteria:

- having diabetes for 1.5 years or less by September 1977	25
- newly diagnosed between September 1977 and September 1978	11
- referred by pediatrician and back to pediatrician	1
	37.

Newly diagnosed patients and children with a duration of 1.5 years or less may have (remnant) endogenous insulin secretion, which facilitates the achievement of good metabolic control. Such achievement would not be due to the home-care system employed.

The changes in admission-days and acute visits (table III of paper 1 - page 199) before and after home-care was introduced were plotted against the number of all children attending the diabetes clinic. Table 4.1 of the present discussion indicates the numbers of children entering or leaving the diabetes clinic, during the follow-up.

Table 4.1. Flux of patients seen at the Sophia Children's Diabetes Clinic.

	September 1977 September 1978	September 1978 September 1979	September 1979 September 1980	September 1980 September 1981	
All children seen	78	88	99	106	
Newly diagnosed	11	10	12	8	in
Referred with treated diabetes by pediatrician (n=19)	1	7	7	4	in
Referred back to pediatrician (n=10)	0	2	7	1	out
Referred to internist (n=11)	0	3	4	4	out
Moved out of area (n=2)	0	1	1	0	out
"Drop-outs" (n=2)	2	0	0	0	out
	"hospital-based"		"home-care"		

Children leaving the diabetes clinic ("out" in the table above) comprised:

- 10 children referred back to the pediatrician elsewhere. These 10 children all came from the 19 referred to the clinic between September 1977 and September 1981 by pediatricians elsewhere ("in" in the table above). None of these 10 children referred back was admitted to the hospital, nor did they visit the clinic in emergency situations.
- 11 children were referred to the internist because of their age. Between September 1977 and September 1978 their averaged number of admission-days was 2.0 days, less than the average of 2.4 days for the whole patient-population in that timespan. The same applied for acute visits. Between September 1978 and September 1981 none of these children was admitted nor did they visit the clinic acutely because of metabolic disregulation. Their HbA_1 were 12.2% on the average (with a range of 10 - 16.3%) not different from the HbA_1% of the 41 children of table II of paper 1 - page 198.
- 2 children moved out of the area, without being admitted or visiting the clinic acutely, their averaged HbA_1 being 11.8% and 12.0%.
- 1 patient was excluded from these tabulations: she was referred in November 1979 with an extremely brittle diabetes, necessitating the use of subcutaneous continuous insulin infusion started in July 1980. She was admitted for more than 100 days and her case history has been reported elsewhere (23).

Taken together, there is no evidence for referral bias with regard to admission-days, acute visits or HbA_1% in the conventionally treated children with diabetes mellitus tabulated in table III of paper 1 - page 199. The same reductions in admissions or acute visits for metabolic disregulation were seen with the introduction of the home-care system in the 41 children that were selected for the follow-up of metabolic control, as in all children attending the diabetes clinic.

b. Nature of admissions and acute visits.

The next tabulation (table 4.2 of this discussion) summarizes numbers of children, the nature of their admissions, acute visits before and after home-care, including the number of newly diagnosed children that received initial treatment without being admitted to the hospital.

Table 4.2. Nature of metabolic disregulation before and after home-care.

	September 1977 September 1978	September 1978 September 1979	September 1979 September 1980	September 1980 September 1981
Number of admission-days	160	65	38	8
Number of children admitted	13	5	3	1
Hyperglycemia/ketosis	9	3	2	1
Hypoglycemia	4	0	1	0
Number of acute visits	24	10	2	1
Hyperglycemia/ketosis	10	5	0	0
Hypoglycemia	14	5	2	1
Number of newly diagnosed children	11	10	12	8
Number of newly diagnosed children not admitted for initial treatment	0	0	5	6

After the introduction of home-care the principal reason for admission was decreased consciousness. In cases of hyperglycemia/ketosis, this coïncided with a blood Ph of 7.25 or less including more than 5 percent dehydration by weight. In cases of hypoglycemia, this involved failure of self-administered glucagon and subsequent carbohydrate intake to normalize consciousness. In the first year of the home-care project (September 1978 - September 1979) three of the five acute visits occurred because of hypoglycemia in families that had not yet been trained in the use of glucagon. A similar situation led to admission in two of the three admissions for hyperglycemia/ketosis in that year: these two families called the help of the nurse practioner only 48 hours after the initiation of symptoms.
Not given in the above table are the numerous visits by the nurse practioner, especially during the first year of the home-care, to assist families in actually treating hypoglycemia or hyperglycemia/ketosis at home: the nature and severity of such metabolic disregulations varied too much to allow a classification thereof.

c. Patient-education.

Teaching to patients was both to individual families at home and in "class-rooms" in the hospital.
Home-teaching was mainly on insulin-injections (all patients having diabetes, except the two "drop-outs", were put on insulin injections twice daily once the "honey-moon"-phase was over), self-monitoring and adaptation thereof in individual circumstances (sports, parties etc.).
Class-room-teaching was in five evening sessions held once a year for parents of whom the children were first seen during that year. By this sequence class-room teaching reinforced the individual lessons taught at home. The evening sessions contained an intermission of of half an hour, during which parents got to know each other. In the first two years of home-care separate evenings were held for the parents of the other children, that were not newly diagnosed during that year.

The program was:

1st evening	a. causes of diabetes; b. insulins, insulin action.
2nd evening	a. self-monitoring (also practical); b. special situations to use self-monitoring.
3rd evening	a. diet and dietary allowances; b. insulin, self-monitoring and diet.
4th evening	a. inheritance, chronic complications, choice of profession; b. pregnancy, anti-conception, future developments.
5th evening	a. multiple choice examination; b. results of examination, final discussion.

Part a. (before the intermission) took three quarters of an hour, part b. (after the intermission) also, including one quarter for questions and discussion. The setting was informal, not involving more than 35 parents per class. The attendance-rate was 70 - 80 percent of the parents invited. Care was taken not to expose parents of very recently diagnosed children to this program, but to advise them to participate in the next years class.
This examination is reprinted next.

Exam for parents of children having diabetes mellitus

All questions are multiple choice. You are kindly requested to darken the circle left of the right answer.

Example:
Diabetes of childhood is caused by taking too much sweets.
a. 0 partially correct
b. ● not correct
c. 0 correct

b = right answer.

---

1. A "hyper" (high bloodsugar) is caused by ...
   a. 0 too little insulin-effect
   b. 0 too much insulin-effect
   c. 0 too little food
   d. 0 a + b

2. Glucose (the main energy source of the body), in case of too little insulin-effect, is ...
   a. 0 not taken up sufficiently by body-cells and subsequently utilized to yield energy
   b. 0 converted into ketones
   c. 0 taken up rapidly by body cells and subsequently utilized to yield energy

3. Ketones (acetone) appears in the urine, if ...
   a. 0 too much fat is broken down, because there is too much insulin-effect
   b. 0 too much fat is broken down, because there is too little insulin-effect is.
   c. 0 none of these (a + b)

4. A child with diabetes may have high bloodsugars (or urinesugars) also if the child eats little or nothing. This is because ...
   a. 0 the body will produce glucose from the liver, if there is too little insulin-effect
   b. 0 food is not carbohydrates only and the body can make glucose from other nutrients
   c. 0 both answers (a + b) are correct

5. The following values of a home-made bloodglucose (day + night) profile are within the "diabetes-norm":
   a. 0 2.2 - 22.2
   b. 0 4.4 - 10.0
   c. 0 4.4 - 13.3

6. Reading the Hemoglucotest (Chemstrip) you will note by preference?
   a. 0 2.2 - 4.4
   b. 0 2.2
   c. 0 4.4

7. The strip for urinary ketones is called?
   a. 0 Diabur
   b. 0 Hemoglucotest (Chemstrip)
   c. 0 Ketur

8. The new strip for urinary glucose can read?
   a. 0 until 5 grams per 100 ml urine
   b. 0 until 2 grams per 100 ml urine
   c. 0 until 10 grams per 100 ml urine

9. Using Hemoglucotest (Chemstrip) the use of a stopwatch is ...
   a. 0 desirable
   b. 0 necessary
   c. 0 cumbersome

10.Glucagon-injections are used in case of ...
   a. 0 low bloodsugar
   b. 0 low bloodsugar if the child cannot or will not take sugar
   c. 0 with vomiting

11.Glucagon is injected ...
   a. 0 like insulin
   b. 0 in the muscle
   c. 0 both answers (a + b) are correct

12.Glucagon will ...
   a. 0 raise the bloodsugar
   b. 0 raise the bloodsugar within 10 minutes
   c. 0 raise the bloodsugar within 10 minutes, but the child must eat subsequently and have bloodsugars measured

13.Insuline Actrapid works ...
   a. 0 12 hours
   b. 0 6 hours
   c. 0 24 hours

14.Insuline Mixtard is ...
   a. 0 1/3 short acting, 2/3 long acting
   b. 0 1/2 short acting, 1/2 long acting
   c. 0 2/3 short acting, 1/3 long acting

15.The most common cause of swinging bloodsugars is ...
   a. 0 eating too much
   b. 0 irregular resorption from the injection site
   c. 0 irregular meal-times

16.The most common cause of fat-pads is ...
   a. 0 injecting too superficially
   b. 0 injecting at one spot
   c. 0 both answers (a + b) are correct

17. A five year old child wakes up and is drowsy, nauseated, refuses breakfast. What would you do?
    a. 0 take the body-temperature, weight and test for urinary ketones and call the hospital
    b. 0 give the usual insulin-dose and do as under a
    c. 0 give sugarwater and do as under b + a
    d. 0 take bloodsugar and do as under a

18. This child has diabetes for one half year and takes 18 U of insulin per day in the morning only. The bloodsugar is 13.3-22.2 and urinary ketones are 2+. No fever. The doctor/nurse advises to give 4 U Actrapid insulin extra. What would you do next?
    a. 0 check every urine for glucose and ketones
    b. 0 check bloodsugar and urinary ketones some 4 hours later and call back.
    c. 0 go to the hospital

19. Another child, 10 years old, wants to participate in an evening running-match over 5 miles. She takes 24 U of insulin in the morning and 8 U in the evening. What would you do?
    a. 0 give extra food before the match
    b. 0 as a, but give 4 U instead of 8 U
    c. 0 as a + b and check bloodsugars before and after the match

20. The following week she has a barbecue-party at 20.00 hours. What would you do?
    a. 0 give 12 U instead of 8 U at her usual dinner time (± 18.00 hours) and have her take her usual dinner
    b. 0 give the usual 8 U at ± 19.30 hours and tell her to take as much food at the party as she would at dinner time
    c. 0 give her 12 U at ± 19.30 hours if she wants to eat more at the party than at dinner, after explaining her how to do this

21. This same girl is on summer-vacation in Southern-France. It is warm and she runs, swims the whole day. What do you suspect?
    a. 0 that less insulin will be needed
    b. 0 as a and try this by taking morning and late afternoon bloodsugars

During the intermission of the last evening the answers were checked, the number of correct answers indicated and the forms returned to the parents. The average percent of acceptable answers was 85 percent, with an annual range from 70 - 92 percent. Answers to questions on materials and their use (insulin, bloodglucosestrips) were almost invariably correct. Mostly also on the (difficult) clinical questions at the end of the examination. Interestingly, most errors were in answers on questions on insulin action, apparently by their abstract nature.

d. Self-monitoring.

The primary aim of self-monitoring was avoidance of hypoglycemia and symptomatic hyperglycemia. The secondary aim of self-monitoring was to get an impression of bloodglucoses during a given day-and-night, taken four times a year. Children and/or parents preferring not to do the latter, got treatment advices on the basis of clinic visits once every 6 - 8 weeks. This included history-taking and the measurement of HbA_1%. This latter category consisted entirely of children of 12 years or older. They constituted about half of the population over that age seen in the first year of home-care. Presently (1983) 24 of 71 of the children of 12 years or older prefer not to note and report their bloodglucoses to the clinic, except under pressing circumstances, such as intercurrent illnesses with impending ketosis. Of these 4 will not visit the clinic at the recommended 3 - 4 monthly intervals. It should be mentioned here that dutch health-care insurance companies, with few exceptions, cover the costs, for 80 - 100 percent, of 25 bloodglucose-strips per month since 1983. Before that time the costs were covered by the home-care project budget and by the separate support of the local national insurance institu-

tion (Stichting Rotterdamse Ziekenfondsen) mediated by its medical advisor Mrs. dr. H. Boers.
An important aspect of self-monitoring of bloodglucose is quality control. Measured in a glucose-reflectometer the linear correlation coefficient of these strips with the laboratory GOD-method on 100 diabetic samples was r = 0.96.
The form used by the families to note self-measured bloodglucoses is reprinted next.

Bloodsugar day-and-night curve

Name ..... Doctor's notes .....
Day and date .....
Insulin dose and sort ..... + ...

n.p.o.	n.p.o.	1 hr after breakfast	before lunch	1 hr after lunch	before supper	24 hrs 3 hrs
Time (marine times)						
Blood-sugar						
Urine-test						
Extra food						
Extra exer-cise						
Hypo's?						
Hyper's?						

Note: it is assumed taking the meal takes not more than 20 minutes.

The day-and-night curves of 15 families, were time-labelled by the families and stored in the container of the bloodglucose-strips, that has an exsiccator. In the hospital these were read again within 48 hours and compared to the values reported by the families. This involved 105 bloodglucose-strips in total. The bloodglucose-strips used (Hemoglucotest 20/800^{R}, Boehringer, Mannheim, F.G.R.) are read in ranges: 1.1 - 2.2 mMol/L, 2.3 - 4.4 mMol/L, 4.5 - 6.6 mMol/L, 6.7 - 10.0 mMol/L, 10.1 - 13.3 mMol/L, 13.4 - 22.2 mMol/L glucose. The fifteen families were selected in case of doubt of the quality of their bloodglucose-assessments. The following deviations were noted with these 105 strips:

- failure to note two rather than one glucose-value as the estimate of bloodglucose, but otherwise agreement — 25 x
- hospital reading off one range of estimate by patient (all at 3 A.M.) — 3 x
- not readable — 4 x
- hospital reading off more than one range of estimate by patient — 0 x
- skipped time-points of day-and-night curve (6x at 3 A.M.) — 30 x

e. Metabolic control.

During the hospital-based period the assay of glycosylated hemoglobin (HbA_1) was not available, hence a comparison between glycemic control achieved by hospital-based and by home-care with this parameter is not possible.
During the three years of the home-care project total glycosylated hemoglobins (t-HbA_1%) were measured (table II of paper 1 - page 198). The findings of paper 2 however question the signifi-

cance of this measurement. Therefore no statistic was used to examine possible changes in t-HbA_1% during the home-care.
With the limitations of HbA_1% as an index of glycemia in mind, the self-measured bloodglucoses of the 41 children (table II of paper 1 - page 198) were examined. This method was introduced with the home-care system, hence only changes during home-care were evaluated.
The following scoring-system was designed, using the bloodglucose-strips described earlier.

Result of self-measurement glucose mMol/L	Points
$\leq$ 1.1	3
1.2 - 2.2	2
2.3 - 4.4	1
4.5 - 6.6	0
6.7 - 10.0	0
10.1 - 13.3	1
13.4 - 22.2	2
$\geq$ 22.2	3

As indicated earlier almost half of all children seen of 12 years and older preferred not to produce bloodglucose day-and-night profiles at each 3-4 monthly visit.
38 of the 41 children chosen for the follow-up (that were much younger, see table II of paper 1 - page 198) had home-profiles made at the frequency intended throughout the three years of the follow-up of home-care. Only if the 3 A.M. value was included and contained seven or more time-points measured in total, their profile was used to calculate "points" according tot he above scoring-system. The parents were instructed to avoid days with intercurrent infections, and unusual physical exercise. The families produced three or more of such profiles during each year of the home-care. The "points" of each child's profile were counted and its summation averaged per child per year of home-care. This is represented in the following tabulation.

	First year	Second year	Third year
Scoring points (means and range)	4.18 (3 - 10)	3.74 (2 - 10)	2.66 (1 - 8)
% lower than 4.4 mMol/L	4	6	9
% higher than 10 mMol/L	43	37	24

To clarify the significance of this scoring-system: 2 scoring-points implies that 5 of 7 glucose-measurements of a given profile were between 4.4 and 10.0 mMol and that 2 measurements were one range below or above this value. In the last year of the project and thereafter (1982 - 1983) many curves were seen with a one-hour post breakfast-value of 10 - 13.3 mMol/L and a 3 A.M. value of 2.1 - 4.4 mMol/L, the remaining values between 4.4 and 10.0 mMol/L. This does not necessarily imply improved overall metabolic control. The trend indicates only that parents, along with home-care, were able to arrive at improved bloodglucose-profiles.

f. Economy of home-care.

The costs of the home-care during 1980 were in Hfl.*:

Nurse-practioners-salary + social securities	60,000.--
Nurse-practioners-transport	10,000.--
Telephone (hospital + parents) - estimated	2,000.--
Strips for selfmeasuring glucose-ketones	10,000.--
Secretarial help, printing, mailing	8,000.--
	90,000.--

* 1 Hfl. = 1 dutch guilder equals about 30 (US) dollar cents

The hospital-costs during home-care in 1980 were in Hfl.:

1 acute visit (hospital + parents) - estimated	400.--
8 admission-days for treated diabetes (1 hospital-day in 1980 costed 400)	3,200.--
26 admission-days for 8 newly diagnosed diabetic children (26 x 400 = 10,400)	10,400.--
	14,000.--

In 1980 106 children attended the diabetes clinic and a total of Hfl. 104,000.-- was spent or Hfl. 980.-- per child.

The costs of the hospital-based-care during 1977 were in Hfl.:

Telephone (hospital + parents) - estimated	1,000.--
Fractioned 24 hrs urinary glucose- and ketone-determinations n = 42	
a. transport by parents - estimated	5,000.--
b. laboratory costs	10,000.--
	16,000.--

The hospital costs during hospital-based-care, based on 1980-norms were in 1977 in Hfl.:

24 acute visits (hospital + parents) - estimated	9,600.--
160 admission-days for treated diabetes	64,000.--
209 admission-days for 11 newly diagnosed diabetic children corrected to 152 days for 8 (the number of newly diagnosed children in 1980)	60,800.--
	134,400.--

In 1977 78 children attended the diabetes clinic and a total of Hfl. 158,400 was spent or Hfl. 2,030.-- per child. In conclusion,

in the same clinic the costs per diabetic child were two-fold lower with home-care than with the hospital-based-system-care. The savings by decreased admission-days for newly diagnosed children were almost as high as those by savings on admission-days for treated diabetes.

The Netherlands have a national hospital registry (Stichting Medische Registratie in Utrecht), which collects all admissions to 95 percent of all hospitals in the Netherlands. The admissions are distinguished by principal and primary (or secondary) diagnoses e.g. diabetes mellitus (principal diagnosis), metabolic disregulation (primary diagnosis). The sex and age of the patient are noted as well as the duration of each admission. Data were purchased from the Stichting Medische Registratie for 0 - 16 year old children with the abovementioned principal and primary diagnosis:

Nation-wide admission-days (1978-1980)
Diabetes mellitus, metabolic disregulation, 0 - 16 years.

1978	19,800
1979	21,100
1980	20,900
	61,800
or	20,600 days per year averaged.

The average duration for diabetes mellitus/metabolic disregulation - first admission - was 21 days. This figure compares well to the 19 days in 1977 during hospital-based-care in our clinic, spent by newly diagnosed children for initial treatment. For the nation-wide incidence study (Chapter II) an ascertainment corrected number of newly diagnosed children (0 - 16 years of age) during those same years of 1,320 children was found, or 440 children per year. The nation-wide number of admission-days for initial treatment may be estimated at 440 x 21 days per child: 9,240

days. By substraction of this number from the total number of admission-days (20,600) the annual number of admission-days for metabolic disregulation after initial treatment may be estimated: 20,600 - 9,240 = 11,460 days.
With an estimated prevalence for diabetes (0 - 16 years) of 3,000 (Chaper II) it follows that each diabetic child would be admitted for metabolic disregulation after initial treatment for almost 4 days per year on the average! This is even higher than the 2.4 days per diabetic child found during hospital-based-care in the Sophia Children's Hospital in 1977 (table III of paper 1 - page 17).
With these nation-wide data on all admission-rates, the costs were 20,600 x 400 = Hfl. 8,36 million in 1980. The savings by a (hypothetical) nation-wide introduction of the home-care-system would comprise Hfl. 4 million if only the savings observed in the Sophia Children's Hospital were extrapolated. From this amount of money alone, some 40 home-care-projects could be maintained, each costing Hfl. 100,000.-- per year.
By assigning 100 children to each project, the total prevalence of childhood diabetes in the Netherlands (0 - 19 years) would be covered.

§5. Discussion

Large reductions in admission-rates of diabetic patients after reorganization of care to these patients was first reported in 1972 from a large U.S. county hospital (24), followed by other centres for adult patients (25) and children (26). The efficacy of our present home-care system was assessed by comparing admission-rates before and after the introduction of the home-care. Also nation-wide admission-rates were used for this purpose. Nation-wide admission-rates were collected from a central hospital registry, marked by "diabetes mellitus - primary diagnosis, metabolic disregulation - secundary diagnosis". This should exclude for example "diabetes mellitus, appendicitis", but the data purchased from the central registry were not ascertained.

In the Funen County of Denmark (450,000 inhabitants) hospitalizations of all previously ascertained type-1-diabetic patients were followed for eight and a half years by record linkage, but the data were also scrutinized from the medical records themselves by the investigators (27). Age, sex and nature of admissions were recorded. Boys from 1 - 14 years spent 3.62 days per child per year in the hospital for metabolic disregulation of their diabetes, girls 5.90 days. These admission-rates from the Funen County were virtually identical to the admission-rates seen in the Sophia Children's Hospital before the introduction of home-care: 160 days for 78 children with treated diabetes and 209 days for 11 newly diagnosed children, totalling 4.19 days per diabetic child per year (table III - paper 1 - page 17).

The health-care system employed in the Funen County resembled the hospital-based system of the Sophia Children's Hospital very closely (27) and supported the use of the hospital-based data as a reference for the efficacy of home-care indirectly.

Again in Denmark, but in another area, a home-care study very similar to the present one was initiated at exactly the same time as the present one (28). This study was a randomized prospective investigation involving newly diagnosed patients (mostly adults) to be followed over two years. A 2.4-fold drop in readmission-days was found in patients receiving home-care compared to the

group of patients, not visited at home.
At the same time stable glycosylated hemoglobins in these newly diagnosed patients were significantly better in the home-care group, but not the profiles of home-bloodglucose or -urinary glucose measurements. In the present study an improvement in scores of home-glucose-profiles was found, but glycosylated hemoglobins were found unreliable in children, that were all well beyond their remission-phase.
It should be emphasized however the improvement in bloodglucose-profiles may not have been due to home-care as a system, but rather to the fact that almost all patients were put on a twice daily insulin regimen with the introduction of home-care. Also improved bloodglucose-profiles do not necessarily implicate better overall metabolic control, but they indicate better patient education and ability to arrive at such improvement by patients. No firm conclusions can be drawn with respect to changes in the quality of metabolic control by virtue of the present home-care.
The potential economic savings by home-care, extrapolated from the savings found by reduced admission-rates in the Sophia Children's Hospital appeared large enough to justify wider application of home-care for diabetic children. Yet the calculations used may be invalid. Citing an economist: "You did not save Hfl. 400. per admission-day reduced as hospitals won't close down beds. You saved mainly hospital-meals and -laundry". On another occasion with tenured health-care politicians: ".... nice study ...., but this system affronts present health-care policies attempting to have health-care provided at home by family physicians and local general nurses, away from hospitals". The author wishes to acknowledge the economic, medical and nursing directory of the Sophia Children's Hospital for their offering the nurse practioner of this study a position in their nursing staff to continue home-care. Their decision to do so was effectuated as soon as the funds for the home-care project, kindly donated by the Dutch Children's Stamp Fund and Novo Industri B.V., expired in 1981.
Perhaps home-care is a model for other complex chronic disorders of childhood.

§6. Summary

The findings of Chapter III may be summarized as follows:

1. Home-care as a means of health-care delivery to children with diabetes mellitus leads to sustained marked reductions in admission-rates and acute out-patient visits.

2. The commitment of an expert nurse-practioner, willing to work also outside regular hours, was crucial to the home-care-system.

3. Commonly used methods to measure glycosylated hemoglobins appeared of very limited practical usefulness in the assessment of glycemic control of long-standing childhood diabetes with poor control.

4. Glucose-determinations obtained by home-monitoring, reflecting the educational status of parents of pre-adolescent diabetic children, improved during home-care.

5. The home-care-system was at least twice less costly as the hospital-based system and deserves wider application in the Netherlands.

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Summary and conclusions

This thesis contains investigations into three aspects of childhood diabetes: etiology (chapter I), epidemiology (chapter II) and therapy (chapter III). The cause (etiology) of the disease is unknown, the incidence (epidemiology) has not been investigated systematically in the Netherlands, the treatment (therapy) is unsatisfactory: the burden for the child and its family is large. The long-term prognosis is not good. The so-called chronic complications emerge after some 20 years of treatment and shorten the life-expectancy by one-third in years.

It has not been established to what extend meticulous treatment will postpone the chronic complications. Apart from the mortality, the morbidity by the chronic complications is considerable. Diabetes mellitus is the leading cause for new cases of blindness; terminal renal-insufficiency is seen 11x more frequent than in the normal population; ischemic necrosis with amputation of bodylimbs 9x; myocardial infarction 7x. All of this next to an array of other invalidating abnormalities.

It is hoped for the studies of chapter I will contribute to a future immuno-prevention of this disease. In the first paper the role of immunogenetic factors, taking part of the major histocompatibility complex (MHC-complex), is investigated in a combined population- and family-study. The association between HLA and insulin dependent diabetes mellitus is confirmed. The association with HLA-DR3 and HLA-DR4 is particularly strong in children. A predominance of HLA-DR4 is found in children younger than 8 years of age. From the family-data the susceptibility to the disease appears to be connected to genes that are in "linkage-disequilibrium" with HLA-haplotypes. The data disagree with either simple recessive, intermediate or dominant Mendelian inheritance. Assuming HLA-haplotypes themselves are linked to the susceptibility to the disease, several forms of segregation can be tested. With this approach the haplotype combinations HLA-B15/-DR4 and HLA-B8/-DR3 appear to contribute separately to the susceptibility to the disease. According to this model, in combination with other findings in the study, the susceptibility to

diabetes is best explained by the effects of one or more genes situated between the HLA-B- and HLA-DR-loci. Also the possible linkage between non-HLA immunogenetic factors and childhood diabetes is examined: the Gm-markers investigated are however not linked to the disease.

The second paper describes a diagnostic application of HLA-serology. A patient with diabetes in the first week of life is presented, which is extraordinary rare. This child has nor HLA-DR4 or HLA-DR3 and lacks antibodies towards the islets of Langerhans, confirming the premise of an unusual pathogenesis of its diabetes. Further examination reveals this child has features of the fetal alcohol syndrome. An association between insulin dependent diabetes and fetal alcohol syndrome has hitherto not been reported.

The third paper explores another hallmark of childhood diabetes: the transient occurence of antibodies against the islets of Langerhans, that normally produce the insulin. A previously established method to estimate cytoplasmatic islet-cell fluorescence predicts insulin dependency in children, before any clinical or biochemical sign of diabetes is present. These antibodies were present 24 and 4 months in two children before symptoms develop. A third child has islet-cell membrane antibodies 32 months before the disease becomes manifest and has cytoplasmatic antibodies thereafter.

The fourth paper examines the nature of cytoplasmatic islet-cell antibodies before and after the disease becomes manifest. Their complement fixing capacity is studied, as other investigators suggest this capacity prevails before the disease becomes manifest and disappears thereafter. This sequence of events is not confirmed. Rather the assessment of complement fixing appears to depend on the sensitivity of the technique employed. Also, the demonstration of IgG-subclasses involved depends on the technique: the higher the titre, the more IgG-subclasses are detected. Two children however have high titres and IgG_2 only and also have a family history of diabetes.

So far the autoimmunity is examined mostly before the manifest disease prevails and insulin treatment is necessary. A pecularity

of insulin dependent diabetes is the so-called "temporary remission": just after the initiation of insulin therapy the doses required often diminish in association with a temporary recovery of the islets of Langerhans to produce insulin. It is not clear why this remission in insulin requirements is temporary or why subsequently higher doses of insulin are needed. It is possible the insulin injections raise antibodies to the insulin receptor. In mice the injection (vaccination) with insulin causes the generation of antibodies towards the receptor for insulin.
In the fifth paper the occurrence of insulin receptor antibodies in man is confirmed. Unexpectedly, these antibodies are also found before the initiation of insulin therapy. This suggests the insulin itself may be part of the disease process. The original hypothesis, the emergence of insulin receptor antibodies as the result of insulin injections holds for two of five children investigated. The effects of their insulin receptor antibodies are tested on rat-fat-cells. Their (paradoxical) lipogenic effect is confirmed. It is of note that these two children develop obesity, not easily explained from their insulin doses nor from their diets prescribed. The complexity of humoral antibodies relevant to childhood diabetes is elaborated in the discussion to chapter I. At the end of this discussion the significance of this complexity is explored with regard to the findings of preliminary studies with immunosuppression in newly diagnosed diabetes of childhood.
Chapter II contains an incidence study of diabetes mellitus among children aged 0-19 years, over all the Netherlands during 1978-1981. The data are obtained retrospectively by questionnaires among all consultant dutch pediatricians and internists and ascertained by gathering the same information among (parent-)members of the patient-organisation (the Diabetes Vereniging Nederland). After correction for ascertainment-percentages, almost 500 new cases are diagnosed each year and the number of prevailing 0-19 year olds with diabetes is estimated at more than 4000.
These data are used in the discussion of chapter I to calculate the logistics of a possible screening for childhood diabetes before the disease is clinically manifest in sibs of children that

already have the disease.
The data are also used in the discussion of chapter III to calculate the economy of "home-care" for diabetic children. The seasonal variation in the incidence of childhood diabetes is used in the literature to suggest a role for viral infections with the clinical onset of the disease. In this dutch epidemiologic survey this variation is hardly seen; only in children that are older than 10 years of age when insulin is first administered, the incidence is higher in the autumn and winter months, than it is in the spring and summer months.
In the discussion the literature on the role of viral infections as causative agents is explored. A pathogenetic mechanism other than viral infections is supposed.
Chapter III concerns the treatment of childhood diabetes. First, past and present trends in the literature over the past ten years are reviewed. Many of these changing concepts are incorporated in a new approach to the health-care delivery to the children, named "home-care". This home-care resulted in a rapid decrease of admission-days for metabolic disregulation of the treated diabetes as well as in emergency visits. Because of this preliminary finding, the original randomized prospective design to study the effects of home-care is interrupted. A follow-up study is reported instead, comparing admission-days and emergency-visits the year before home-care is introduced to three years of home-care thereafter.
The first paper of this chapter briefly reports effects of the home-care: a thirty-fold decrease in both admission-days and emergency-visits is found. Also a marked reduction in admission-days for the institution of insulin-therapy in newly diagnosed children is noted.
The second paper examines the significance of various components of glycosylated hemoglobins as parameters of the quality of the metabolic control of the treated disease. This parameter is also followed during home-care. However, these components of glycosylated hemoglobins appear not to be a suitable parameter in labile forms of childhood diabetes.

Subsequently the shift in patients during home-care is described. The techniques used by families at home to self-measure bloodglucose are evaluated and the patient-teaching program are described. It is calculated home-care is a much cheaper system than prevailing hospital-based health-care delivery: from the savings by home-care 40 projects may be run. If 100 children, aged 0-19 years, are assigned to each project, the total prevalence in the Netherlands is covered. In the discussion the findings with home-care are compared to those in other countries.

Samenvatting en conclusies

Dit proefschrift beschrijft onderzoek over drie aspecten van diabetes mellitus bij het kind: de etiologie (hoofdstuk I), de epidemiologie (hoofdstuk II) en de therapie (hoofdstuk III). De oorzaak (etiologie) van deze ziekte is niet bekend. Het vóórkomen (epidemiologie) is in Nederland nog niet systematisch onderzocht. De behandeling (therapie) is onbevredigend: de belasting voor het kind en het gezin zijn groot. Op langere termijn is de prognose niet goed. De zogenaamde chronische complicaties treden op na gemiddeld 20 jaar behandeling. Deze chronische complicaties bekorten de levensverwachting met een derde in levensjaren. Het is onzeker in hoeverre nauwgezette behandeling deze lange termijn prognose kan beïnvloeden. Afgezien van de mortaliteit, is de morbiditeit door de chronische complicaties aanzienlijk. Diabetes mellitus is thans de meest voorkomende oorzaak van blindheid; op den duur terminale nierinsufficiëntie komt 11x vaker voor dan bij de vergelijkbare normale bevolking; gangreen met amputatie van een ledemaat 9x; hart-infarct 7x. Dit alles naast tal van andere invaliderende afwijkingen.

De onderzoeken van Hoofdstuk I dragen hopelijk bij tot een toekomstige preventieve behandeling van de ziekte.

In het eerste artikel is de rol van genen die deel uitmaken van het major histocompatibility complex (MHC-complex) onderzocht, door middel van een gecombineerd populatie- en familie-onderzoek. De associatie van HLA met insuline afhankelijke diabetes mellitus wordt bevestigd. Vooral wanneer de ziekte zich bij het kind openbaart, is de associatie met HLA-DR3 en/of -DR4 sterk. Bij kinderen jonger dan 8 jaar lijkt HLA-DR4 te overwegen. Uit het familie-onderzoek blijkt dat de gevoeligheid voor het krijgen van de ziekte verbonden is met genen, die in "linkage disequilibrium" verkeren met HLA-haplotypen. De familiegegevens pleiten tegen enkelvoudige recessieve, intermediaire of dominante Mendelse overerving.

Wanneer wordt verondersteld dat HLA-haplotypen zelve met gevoeligheid voor diabetes mellitus zijn geassocieerd, kunnen diverse

vormen van overerving worden getoetst. Met deze benadering lijken de haplotype-combinaties HLA-B8/-DR3 en HLA-B15/-DR4 elk een aparte bijdrage tot de predispositie voor diabetes met zich mee te brengen. Volgens dit model en een aantal andere bevindingen uit dit artikel wordt de predispositie voor diabetes, voor zover geassocieerd met het MHC-complex, het beste verklaard door geneffecten, gelegen tussen het HLA-B en het HLA-DR locus. Naast de immunogenetische factoren van het MHC-complex is de rol van Gm-allotypen bij het ontstaan van diabetes bestudeerd. Deze allotypen blijken niet geassocieerd te zijn met het vóórkomen van diabetes in families.

In het tweede artikel wordt een diagnostische toepassing van HLA-serologie beschreven. Het gaat om een patiënt, die reeds één week na de geboorte diabetes krijgt, hetgeen extreem zelden gebeurt. Dit kind heeft noch HLA-DR3 noch HLA-DR4 waardoor het vermoeden op een afwijkende pathogenese wordt gesterkt. Ook ontbreken antilichamen tegen de eilandjes van Langerhans. Bij nader onderzoek blijkt dit kind verschijnselen van het foetale alcohol syndroom te hebben. Een samengaan van diabetes mellitus en foetaal alcohol syndroom is tot dusverre niet beschreven.

In het derde artikel is een ander hoofdkenmerk van diabetes onderzocht: het vóórkomen van antistoffen tegen de eilandjes van Langerhans, waar insuline normaliter wordt geproduceerd. Een tevoren gestandaardiseerde methode om cytoplasmatische fluorescentie aan te tonen, lijkt diabetes in-statu-nascendi bij kinderen te kunnen vaststellen; dat wil zeggen, vóórdat enig klinisch of biochemisch verschijnsel van diabetes aanwezig is. Bij twee kinderen kon worden aangetoond dat deze antistoffen aanwezig waren 24 en 4 maanden voordat de ziekte zich openbaarde; een derde kind had 32 maanden voordien alleen antistoffen gericht tegen de membraan van eilandjes van Langerhans en vervolgens cytoplasmatische antistoffen, vanaf het moment dat de ziekte klinisch manifest was.

In het vierde artikel is de aard van deze antistoffen onderzocht, zoals die voorkomen bij kinderen bij wie de ziekte zich klinisch heeft geopenbaard; in het bijzonder zijn de complement-fixerende eigenschappen van cytoplasmatische antistoffen onderzocht, dit

omdat andere onderzoekers suggereerden dat deze eigenschap zou verdwijnen bij het klinisch manifest worden van de ziekte. Een dergelijke samenloop bij het klinisch manifest worden van de ziekte wordt niet bevestigd. Daarentegen blijkt dat het vaststellen van complement-fixatie afhankelijk is van de gevoeligheid van de daartoe gebruikte techniek. Ook het aantonen van de IgG-subklasse van deze antilichamen is van de gebruikte techniek afhankelijk: hoe hoger de titer, des te meer IgG-subklassen worden gevonden. Bij twee kinderen met hoge titers van deze antilichamen werden alleen IgG2-antilichamen aangetroffen, waarbij opviel dat insuline afhankelijkheid vaker in hun familie voorkomt dan meestal het geval is.

Tot hier toe is het onderzoek gericht op de auto-immuniteitsaspecten van diabetes, vóórdat de ziekte klinisch manifest wordt en met insuline behandeling moet worden begonnen. Een bijzonderheid in het beloop van de behandeling met insuline is, dat de benodigde dosis na het begin van de behandeling vaak - tijdelijk - zeer veel minder wordt. Dit berust op een herstel van het insuline producerend vermogen van de eilandjes van Langerhans en wordt de "tijdelijke remissie-fase" genoemd. Het is onduidelijk waarom deze remissie tijdelijk is en daarna weer hogere doses insuline nodig zijn. Eén van de mogelijkheden is dat de insuline injecties aanleiding geven tot de vorming van antistoffen tegen de receptor voor insuline. Daardoor zou het lichaam minder gevoelig kunnen worden voor eigen of toegediend insuline, waaruit een hogere insuline behoefte voortvloeit. Deze veronderstelling stoelt op experimenten bij muizen, waarbij de inspuiting (vaccinatie) met insuline antistoffen tegen de insuline receptor opwekt.

In het vijfde artikel is het vóórkomen van dergelijke antistoffen bij de mens beschreven. Een onverwachte vondst bij dit onderzoek was dat zulke antistoffen ook worden aangetroffen bij kinderen, vóórdat insuline wordt geïnjecteerd. Daarmede wordt de mogelijkheid geopperd dat insuline zelf bij de auto-immuniteit betrokken kan zijn, als oorzakelijke factoren in het ziekte-proces. De oorspronkelijke veronderstelling, het vóórkomen van antistoffen tegen de insuline-receptor volgend op insuline-therapie, is bewaarheid bij 2 van de 5 daarop onderzochte kinderen. Deze receptor

antistoffen worden wat hun effect betreft getest op rattevetcellen en blijken een (paradoxaal) lipogeen effect te hebben. Het is opmerkelijk dat juist deze twee kinderen beiden vetzucht ontwikkelden, die niet goed kan worden verklaard uit de door hen gebruikte insuline-doses, noch uit het voorgeschreven dieet.
In de discussie bij hoofdstuk I wordt de complexiteit van de rol van humorale antistoffen bij diabetes toegelicht, naast de tekortkomingen van de thans beschikbare bepalingstechnieken.
Tenslotte wordt ingegaan op de betekenis van elders lopende onderzoeken met immuno-interventie bij pas ontdekte diabetes, tegen de achtergrond van bovengenoemde complexiteit.
In Hoofdstuk II is een incidentie-bepaling (het vóórkomen van nieuwe gevallen) beschreven van diabetes mellitus bij kinderen van 0 - 19 jaar, over geheel Nederland gedurende de jaren 1978 - 1981. De gebruikte gegevens zijn verkregen door middel van een enquête die werd gehouden onder alle klinisch werkzame internisten en kinderartsen in het land. De betrouwbaarheid van dit retrospectieve onderzoek is onderbouwd door het apart opvragen van dezelfde gegevens bij leden van de patiënten-vereniging, de Diabetes Vereniging Nederland. Wanneer met de onbetrouwbaarheid van de gegevens uit de specialisten-enquête wordt rekening gehouden, blijken gemiddeld bijna 500 nieuwe gevallen per jaar te zijn vastgesteld. Het aantal bestaande gevallen van diabetes mellitus onder kinderen van 0-19 jaar is op ruim 4.000 geschat.
In de discussie van Hoofdstuk II zijn deze incidentie-gegevens gebruikt om logistieke facetten van een eventuele screening op voorstadia van diabetes mellitus bij broers en zusters van kinderen, die de ziekte al hebben, te berekenen. In de discussie van Hoofdstuk III zijn de gegevens gebruikt om economische aspecten van "thuisbehandeling" te belichten.
Het seizoengebonden vóórkomen van nieuwe gevallen van diabetes bij kinderen wordt in de literatuur gebruikt als aanwijzing voor een rol van virale infecties bij het manifest worden van de ziekte. In dit Nederlandse onderzoek is deze tendens maar zeer gedeeltelijk waarneembaar; namelijk alleen bij kinderen die ouder dan 10 jaar zijn, wanneer hun eerste insuline-injectie wordt toegediend.

In de discussie werd de literatuur over de rol van virale infecties bij het ontstaan van de ziekte nader onderzocht. Een ander pathogenetisch mechanisme dan virale infecties wordt geopperd.
Hoofdstuk III gaat over de behandeling van kinderen met diabetes mellitus. Eerst wordt omschreven op welke manier de opvattingen hierover zijn veranderd gedurende de afgelopen tien jaar. Veel van deze veranderde opvattingen zijn terug te vinden in een nieuwe vorm van behandeling, die thuisbehandeling wordt genoemd. Deze aanpak leidde tot een zeer snelle vermindering van het aantal opname-dagen en het aantal acute polikliniek-bezoeken wegens ontregeling van de diabetes. Om die reden moest worden afgezien van de oorspronkelijke opzet van onderzoek naar de resultaten van thuisbehandeling. Deze oorspronkelijke opzet betrof een vergelijkend onderzoek gedurende anderhalf jaar tussen thuisbehandeling enerzijds en de gebruikelijke behandelingswijze anderzijds. In plaats daarvan is een vervolg-onderzoek gedaan naar effecten van thuisbehandeling.
In het eerste artikel van dit hoofdstuk wordt in het kort beschreven in hoeverre het aantal opname-dagen en acute polikliniek-bezoeken vermindert gedurende drie jaar thuisbehandeling; dit wordt vergeleken met het aantal opname-dagen en acute polikliniek-bezoeken gedurende het laatste jaar voordat met de thuisbehandeling voor alle kinderen tegelijk is begonnen. Na drie jaar thuisbehandeling is er een 30-voudige teruggang opgetreden in het aantal opname-dagen wegens metabole ontregeling en in acuut polikliniek-bezoek. Ook de daling in het aantal opname-dagen nodig voor het instellen van pas ontdekte diabetes is aanzienlijk.
In het tweede artikel wordt de betekenis van diverse componenten van geglycosyleerd hemoglobine, als parameter van de metabole instelling van de diabetes onderzocht. Deze componenten weerspiegelen kortere perioden van glycemie echter niet.
Vervolgens wordt toegelicht hoe de in- en uit-stroom van patiënten voor en gedurende de thuisbehandeling is geweest. De door de gezinnen thuis gebruikte technieken om zelf bloedsuikers te meten en het opleidingsprogramma voor de ouders van kinderen zijn nader uiteengezet. Ook is het verloop van het patroon in thuis gemeten bloedsuikers gedurende drie jaar beschreven.

Thuisbehandeling leidt niet alleen tot veel minder opnames en acute polikliniek-bezoeken, maar is ook aanzienlijk goedkoper: de besparingen uit thuis-behandeling kunnen de kosten van 40 dergelijke projecten dekken. Wanneer ieder project 100 kinderen van 0-19 jaar oud bestrijkt, is de totale prevalentie in Nederland omvat.

In de discussie van hoofdstuk III worden de bevindingen met thuisbehandeling naast die in het buitenland geplaatst.

Nawoord

Dit proefschrift kwam voort uit samenwerkingsverbanden, zoals blijkt uit het auteurschap van de opgenomen artikelen.
Ik wil hier mijn dankbaarheid betuigen aan vele anderen die hun bijdrage hebben geleverd. Ik beperk mij echter tot de direct bij het werk betrokkenen, wetend daarmee anderen, die daartoe bijdroegen, te kort te doen. Ik hoop dat zij mij in de gelegenheid willen stellen hen op een andere wijze te bedanken.
Prof. Dr. H.K.A. Visser, mijn opleider en promotor, heeft de omgeving, of beter, het geestelijk klimaat geschapen, waarin dit proefschrift kon worden bewerkt. Dit lijkt een vanzelfsprekendheid. Dat is het niet wanneer men zich bedenkt, hoe complex de eindverantwoordelijkheid is voor optimale behandeling, onderzoek en onderwijs met name ten aanzien van zieke kinderen. Dit geldt zeker in een tijd waarin de mogelijkheden daartoe snel veranderen.
Prof. Dr. J.J. van Rood, mijn co-promotor, dank ik voor de diepgaande belangstelling welke hij heeft voor de ziekte, waarover dit proefschrift gaat. Het vertrouwen dat hij ons samenwerkingsverband schenkt vormt een zeer bijzondere stimulans.
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I would like to acknowledge Dr. G.B. Forbes of the Rochester Medical School, who lead my first steps in the area of childhood diabetes.
Prof. Dr. J.L. van den Brande bracht mij de beginselen van de kinder-endocrinologie bij en maakte mij vertrouwd met laboratoriumtechnieken.
Een proefschrift bewerken gaat altijd ten koste van dagelijkse werkzaamheden, in het bijzonder die van de diabetes-polikliniek. Deze polikliniek is een team-gebeuren en dit functioneert alleen wanneer de daarbij horende geest aanwezig is. Deze geest werd op onnavolgbare wijze vertegenwoordigd door:

Mw. Zr. J.J. de Visser, verpleegkundige, Mw. A.E. Hart, diëtiste, Mw. E. Berkhouwer, secretaresse, Mw. C.E. de Beaufort en Mw. C.M.G.J. Houtzagers, artsen, Mw. Dr. F.M.E. Slijper, psychologe, R.S.R. Aarsen, kinderarts, H.J. Aanstoot, arts, R.B. Minderaa en F.C. Verhulst, kinderpsychiaters. Hen is veel dank verschuldigd voor hun aanhoudende steun.

De diabetes-polikliniek maakt deel uit van de polikliniek voor endocrinologie en groeistoornissen. Veel is ook te danken aan de stimulans van Dr. S.L.S. Drop. Prof. Dr. H.J. Degenhart gaf waardevolle adviezen en beheerde het laboratorium. De Heer J.B.C. van Veen, directeur van de medische afdeling van Novo Industri B.V. te Amsterdam ondersteunde het onderzoek met literatuurgegevens. Mw. A. Aanstoot-Brandsma typte het gehele manuscript, nadat Mw. M. de Bruyne daarmee een aanvang had gemaakt.

De beide paranymfen, Dr. C. Pries en Dr. R.J. in 't Veld met hun echtgenoten en kinderen zijn door jarenlange vriendschap verbonden met de verdediging van het proefschrift en bijbehorende stellingen.

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Mijn vrouw Wanda en onze kinderen Iris en Hilgo voltooiden deze periode met wijsheid. Ik verheug mij op onze vakantie.

Curriculum vitae

De schrijver van dit proefschrift werd op 15 juli 1941 te Eindhoven geboren. Nadat hij in 1960 aan het Lorentz-lyceum te Eindhoven het diploma gymnasium bêta behaalde, werd begonnen met de studie van de geneeskunde te Leiden met een beurs van het Philips-van der Willigen fonds.
De stage pathologische anatomie werd aan de Royal Infirmary te Edinburgh gevolgd; een maand lang was hij werkzaam aan het Hôpital des Maladies Contagueuses te Parijs. Als lid van het studentenfaculteitsbestuur voerde hij in 1965 een oriënterend onderzoek uit naar de appreciatie door studenten van het geboden candidaatsonderwijs, gecoached door wijlen Prof. Dr. C. Vervoort, onderwijssocioloog te Leiden.
In 1968 studeerde hij af en werd hij als districtspsychiater toegevoegd aan het Territoriaal Bevel Zuid van de Koninklijke Landmacht onder leiding van Dr. L. Somers en Dr. D. Mulder. Tevens werkte hij op de afdeling Anthropogenetica te Leiden (Prof. Dr. M. Siniscalco).
In 1969 werd hij aangenomen als assistent-kinderarts in opleiding bij Prof. Dr. H.K.A. Visser, in het Sophia Kinderziekenhuis te Rotterdam.
In 1973 werd een vrije stage in de kinderendocrinologie gevolgd, geleid door Prof. Dr. J.L. van den Brande.
Van eind 1973 tot begin 1977 was hij werkzaam als NIH-research fellow onder Prof. G.B. Forbes aan de University of Rochester Medical School, Rochester (N.Y.), USA, het laatste jaar toegevoegd aan de staf als instructor of pediatrics. In die jaren werkte hij met Prof. I.B. Pless over diverse aspecten van diabetes mellitus bij kinderen als model voor chronische ziekte.
In 1976 werd hem de Rochester Diabetes Award (van de American Diabetes Association) overhandigd door wijlen Prof. A. Lazarow.
In 1977 keerde hij terug naar het Sophia Kinderziekenhuis en was hij werkzaam bij Prof. Dr. J.L. van den Brande.
In 1978 werd een aanvang gemaakt met de in dit proefschrift beschreven onderzoeken.
Intussen kreeg hij zitting in de medische subcommissie van de

Diabetes Vereniging Nederland (DVN), werd hij lid van de Werkgroep Diabetes Mellitus bij kinderen van de Nederlandse Vereniging voor Kindergeneeskunde en was hij formeel oprichter van de Diabetes Education Study Group-Netherlands.
De schrijver is gehuwd met Wanda Hartland. Zij hebben twee kinderen, Iris en Hilgo.