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Glycaemic control: evaluations of HbA1c as a risk factor and the effects of modern insulins in clinical practice

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Glycaemic control: evaluations of HbA1c as a risk factor and the effects of modern insulins in clinical practice

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Abstract

One of the ultimate goals of diabetes care is to minimise diabetic complications. When evaluating insulins it is important to understand what extent of improvements in glycaemic control is clinically relevant in preventing diabetic complications. We have thus both studied the effects on glycaemic control of the most commonly used insulins and the relations between glycaemic control and diabetic complications.

In analyses of electronic tracking of patient record systems and data from the landmark study Diabetes Control and Complications Trial (DCCT) were used. Research and statistical models were developed to estimate time-dependent effects between HbA1c and diabetic complications.

Patients receiving insulin glargine in clinical practice have decreased on average 0.18 percentage units in HbA1c compared to patients continuing with NPH insulin. Lean men had the greatest reductions in HbA1c. In corresponding analyses of insulin lispro reductions of HbA1c by 0.19 percentage units were achieved compared to patients continuing with regular insulin. Patients with high HbA1c experienced the greatest reductions in HbA1c.

When relating HbA1c to diabetic complications we introduced the term HbA1c-variable describing different weightings and combinations of HbA1c values. In a systematic review we found that the baseline value was most common to use in studies of HbA1c and diabetic complications, but a mean value of many HbA1c values had greater predictive ability. By simulations we showed that HbA1c-variables comprising time-dependent effects of HbA1c could have 100% greater predictive power than a mean value. In the DCCT we could confirm these results and describe the temporal relationship between HbA1c and retinopathy. Over 6 years an HbA1c-level of 8% instead of 7% predicted 92% greater risk of retinopathy when time-dependent effects were considered instead of 50% with a mean value. HbA1c values 2.4 years ago had the largest deleterious effects on current risk of retinopathy and historical values up to 5 years ago were more harmful than present values. The current salutary effect of a constant lower level of HbA1c increased steadily with time since both present and previous values reduce the current risk of retinopathy. When lowering HbA1c from 9% to 7% 274 patients had to be treated during the first 3 years after diagnosis, but only 2 patients during the period 9-12 years to prevent retinopathy. With time also relatively small HbA1c changes of 0.3% showed a low NNT of 13.

In conclusion, good glycaemic control is more important than earlier recognised in preventing retinopathy. Insulin lispro and insulin glargine improve glycaemic control in clinical practice

and the reductions obtained in HbA1c are clinically relevant. In medicine time-dependent effects of treatments and risk factors should be regarded in epidemiologic and clinical trials to understand the magnitude of the effects. Electronical tracking of data in clinical research and quality improvement is more efficient than manual collection, extensive information is retrieved and costs are reduced substantially.

Key words: HbA1c, glargine, lispro, time, retinopathy, clinical practice, electronical tracking, record system

List of original publications

This thesis is based on the following papers, identified in the text by their Roman numerals:

I. Österbrand M, Fahlén M, Odén A, Eliasson B. A method to predict the metabolic effects of changes in insulin treatment in subgroups of a large population based patient cohort. *European Journal of Epidemiology*. 2007;22:151-15.

II. Lind M, Fahlén M, Happich M, Odén A, Eliasson B. The effect of insulin lispro on glycemic control in a large patient cohort. *Diabetes Technology & Therapeutics*. 2009;11:51-6.

III. Lind M, Odén A, Fahlén M, Eliasson B. A systematic review of HbA1c variables used in the study of diabetic complications. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*. 2008;2: 282-293.

IV. Lind M, Odén A, Fahlén M, Eliasson B. The true value of HbA1c as a predictor of diabetic complications: simulations of HbA1c variables. *PLoS ONE*. 2009;4:e4412.

V. Lind M, Odén A, Fahlén M, Eliasson B. A methodological study of the temporal relationship between HbA1c and retinopathy in the Diabetes Control and Complications Trial (DCCT). 2009. *Submitted*.

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Chapter 1 Introduction

1.1 The perspective of the thesis

The prevalence of diabetes worldwide has been estimated at 2.8% for the total population in 2000 and is expected to increase to 4.4% in 2030 (1). The main cause of the increase is that more people will become over 65 years of age. Patients with diabetes are estimated to increase from 171 million people to 366 million people with diabetes. Diabetes constitutes 5-10% of all health economical costs in the western world (2-4). The major part of these costs are due to diabetic complications. Diabetic complications are retinopathy (injuries on the eyes), nephropathy (injuries on kidneys), neuropathy (injury on nerves), ulcers, amputations, stroke and myocardial infarction. One of the ultimate goals with diabetes care today is to minimise the risks for these complications (5). It is well-known that good glycaemic control is of importance in preventing diabetic complications. However, when evaluating effects of treatments it is also important to understand what extent of improvements should be considered clinically relevant in preventing complications. In this thesis we have thus both studied the effects on glycaemic control of the most commonly used insulins today and the relations between glycaemic control and diabetic complications.

Studies from clinical practice of insulin treatments are probably more important than for many other medical therapies. One reason is that patients with insulin treatments are highly interactive with their therapies and when the support and control in clinical trials are lost, it is difficult to know what effects could be expected in clinical practice (6, 7). Furthermore clinical trials of insulin treatments have generally not been blinded leading to risks of both treatment and assessment biases. In the end it is the effects in clinical practice that will affect the burden of diabetic complications arising worldwide. If we do not understand if modern insulins improve glycaemic control in clinical practice, we do not know whether these treatments help to reduce diabetic complications.

Patients in clinical practice are exposed to hyperglycaemia during much longer periods than the time frames of clinical trials. When examining the importance of glycaemic control in preventing diabetic complications it has not earlier been examined how the salutary effect of improving glycaemic control varies with time (Paper III). If the preventive effect of good glycaemic control increases with time, there could be a larger beneficial effect by improving glycaemic control than has earlier been recognised. Any improvements in glycaemic control by modern insulins would then be more important in preventing diabetic complications.

In a historical cohort of 20,000 patients with diabetes we examined the effects on glycaemic control in clinical practice obtained by modern insulins. The large amount of data was possible to collect with the help of electronical tracking of patient record systems (8). This kind of data collection has earlier been used in studies in different medical fields but is relatively speaking a novel approach in research. To understand the importance of the effects on glycaemic control obtained by modern insulins in clinical practice we have also developed research models and statistical methods to estimate temporal relationships between glycaemic control and diabetic complications. Data from clinical practice as well as publicly available data from the Diabetes Control and Complications Trial (DCCT) were used (9). The

DCCT, which originally was presented in 1993, has generated results forming the basis of clinical guidelines in type 1 diabetes (5, 10).

Hence, in this thesis besides the specific diabetic questions a novel approach of collecting research data, development of research models and statistical methods are presented. We first present the historical background of the corner stones leading to the current clinical research paradigm of diabetes and modern clinical care. To better understand the specific aims and design of our studies we then give an overview of present knowledge of earlier studies of the most commonly used insulins today and previous estimations of the relation between glycaemic control and diabetic complications.

1.2 The discovery of insulin

Before the current era of diabetes management it was for a long time puzzling how diabetes at all could be treated and when diabetic complications started to arise how they could be prevented. The term diabetes was already introduced 100-200 B.C., by Demetrius of Apameia meaning “pass or run through” since in people with diabetes intake of fluid just seemed to run through the body (11). In the second century A.D. Erreteus of Cappadocia made the first descriptions of diabetes. He described large volumes of urine, thirst, melting down of fat and muscles pain and a short life course. He believed that the primary cause of diabetes was the kidneys and the urine bladder and that some mechanism in these organs failed to stop producing water. During the same period Galen (129-200 A.D.) described that diabetes originated from the kidneys but showed evidence that the urine bladder was not involved in the pathogenesis of diabetes. This perspective of the kidney as the primary cause of diabetes would last for many centuries.

Both Erreteus of Cappadocia and Galen described diabetes as a rare condition. However, during the next periods of time diabetes was more frequently described and the prevalence and awareness of the disease seemed to increase with time. It took however many years before the first paradigm shift of how diabetes was looked upon happened. Studies of Paracelsus (1493-1541) of evaporations of urine from a patient with diabetes showed an excess of residual urine containing salts. He believed the blood was involved in the pathogenesis of diabetes with increased levels of salts passing through the kidneys. This was a different view than earlier when the kidneys had been looked upon as the primary cause. Thomas Willis (1621-1675) also made evaporations of urine from a diabetes patient two centuries later and he tasted the residual urine and described it “as if it imbued with honey and sugar” which is the meaning of mellitus. Willis therefore described a test for differing diabetes from other forms of polyurias. However, it took more than a century before Willis’s hypothesis of a substance in the blood that is secreted to the urine was confirmed. Robert Wyyett then in 1774 detected saccharine matter in the blood and urine of patients with diabetes and in 1776 Dobsson could quantify the amounts of sugar in the urine. He could also show that the existence of sugar in the urine happened shortly after or simultaneously as sweetness and saccharine matters were evident in the blood, although the levels in the blood were lower than in the urine. In 1815 Michel Eugene Cheereuil could define the sugar in the blood as glucose. The quantitative analyses were improved and in the second half of the 19th century diabetes could be diagnosed through a quantitative analysis of the glucose level in the urine.

Although diabetes at this point of time was seen as a disease in the blood, the kidneys were still considered as an important cause of diabetes during the first half of the 19th century. Better quantitative analyses made it possible to understand that it was increased levels of blood sugar that caused the elevated levels of glucose in the urine and not primarily the kidneys. It led to the hypothesis that diabetes was caused by increased uptake of glucose from the gastrointestinal tract. Hence diabetes was looked upon as a gastrointestinal disease for some period of time. It led to the carbohydrate intake for diabetes patients becoming essential in the treatment of diabetes which would also be the only treatment with some efficacy until the insulin was detected.

During the second half of the 19th century and early 20th century evidence began to appear that diabetes could possibly be an endocrine disorder originating from the pancreas. In 1869 Paul Langerhans described parts looking like islets in the pancreas and acinal cells that secreted digestive enzymes (12). In 1889 it was understood that the pancreas was central in the pathogenesis of diabetes mellitus. Joseph von Mehring and Oscar Minkowski detected that when removing the pancreas diabetes mellitus was developed. Laques discovered in 1893 that a substance for the breakdown of glucose in the blood could be secreted from the pancreas. In 1906 Zuelzer injected pancreatic extract under the skin of a comatous 50-year old diabetes patient in Berlin and there was an apparent initial improvement in the clinical state but the patient later on again fell into a deep coma. By injections of pancreatic extracts Best and Macloud in 1921 succeeded in decreasing blood glucose levels in a pancreaectomised dog.

A young Canadian boy of 14 years of age, Lenard Thompson, was dying in diabetes in 1920-21 in Toronto University Hospital Canada. He had the typical signs of acute ketoacidosis with high levels of blood glucose, smell of acetone and exhaustion. After discussion with Leonard Thompson's father the decision was taken to try the injections of pancreas extractions developed by Macloud and Banting. The extractions were injected in the buttock of Leonard Thompson in January 1922 in Toronto, Canada. The blood glucose and urine levels decreased but there was no effect in the clinical condition. However, the injections continued and day by day Leonard Thompson became stronger and in a better mood and could later go to work with daily insulin injections. He lived another 13 years with the help of these injections until he died at 27 years of age. The importance of insulin in diabetes and its clinical effects was for the first time definitely established. In 1923 Professor John James Richard Macleod and Frederick Grant Banting were awarded the Nobel Prize for the discovery of insulin (12).

Table 1: The perspective of the pathogenesis of diabetes during different time periods

200 A.D.-1775?	Kidney disease Kidneys cannot retrieve substances
1775 – 1850	Kidneys and the blood
1850-1900	Gastrointestinal Disease Too much glucose is absorbed from the gastrointestinal tract
1900-	Endocrine Disease

1.3 The development of modern insulins

After the discovery of insulin by Professor John James Richard Macleod and Frederick Grant Banting the development of injections of different forms of insulins developed rapidly (13). Hoechst had a great importance for further development of insulins. In 1923 they made insulin available for doctors in Germany, set the standard for slow release insulin preparation in 1930 and in 1934 developed a procedure to produce insulin crystals with zinc (12). The zinc-insulins had a much slower release of insulins than regular insulin which is attributable to the fact that when zinc atoms are added to the solution of dimers which constitute regular insulin, the molecules associate and hexamers are formed (13). These large molecules diffuse slowly into the circulation whereas the insulin monomers and dimers are more rapidly absorbed. The prolonged effect profile was necessary to obtain an adequate insulin replacement therapy without many injections per day and zinc-insulin was generally taken once a day.

Also in the 1930's the Danish doctor Hans Christian Hagedorn and B. Norman Jensen found another way of prolonging the effect of regular insulin (12). In 1936 they showed that the effect of insulin can be extended if it is bound to protamine which is a protein from fish sperm (13). After the addition of protamine to insulin the solution had to be brought to pH 7 before injection. The PZI-insulins were then developed which were a molecule combined of insulin, zinc and protamine with the advantage that it did not have to be brought to pH 7 before injections, but only to be shaken. The protamine zinc insulins were developed in the 1930s and set a trend of giving insulin once daily without addition of regular insulin.

In 1946 crystals were possible to form from insulin and protamine and the Neutral Protamine Hagedorn (NPH) insulin was then marketed. The NPH insulin had an intermediate duration of effect and somewhat shorter than the lente-insulin. It had the advantage that it could be mixed with regular insulin to mixinsulin with both a short-acting effect and an intermediate effect. During the next two decades it became more common to use a complete coverage of the insulin requirement with the regime of mixed insulins twice daily and regular insulin.

Through the discovery of insulin by Macloud and Banting in 1922 the shape of the disease diabetes mellitus also changed dramatically from primarily an acute disease to become a chronic disease. Earlier on the usual survival of disease was a few months from diagnosis for children and young adults with diabetes (14). For older patients the life-course was somewhat longer but the prognosis also very poor. When the insulin treatment was not available the glucose levels could not efficiently be lowered and patients died from acute complications such as ketoacidosis. However, it should be noticed that in many undeveloped countries insulins are today not available and hence the course of diabetes is still primarily an acute disease.

When insulin treatment over time got widely available, diabetes patients lived longer and it became apparent that patients with diabetes received injuries in certain organs such as eyes and kidneys, i.e. diabetic complications. Although chronic diabetic complications did not become evident in clinical practice until the 20th century, signs of retinopathy, proteinuria and neuropathy had been described already during the 18th and 19th centuries (15, 16). It was however not until the 1940s that retinopathy and nephropathy were considered as frequent disorders and specifically related to diabetes mellitus.

In the 1960's another key step was taken in the development of insulins, namely the determination of the chemical structure of the insulin molecule (12). Sanger was awarded the Nobel Prize in

1958 for the structure of 58 amino acids in two chains. The discovery was to be essential for both the synthesis of synthetic insulins and the development of modern insulin analogues. However, for a long time the zinc insulins were still used and NPH insulins are still one of the most commonly used basal insulins in clinical practice (13).

During the early 1980's purified pork insulin and recombinant human insulin to a large extent reduced allergy to insulins and immune mediated lipotrophy. These developments led to the innovation of insulins slowing down. Insulin pumps were also developed and in 1989 the first optipen to facilitate insulin injections was introduced. The first insulin pump that could be programmed was developed in 1990 and in 1993 biosynthesis of human insulin starts.

As diabetic complications became an evident burden for diabetes patients the research increased concerning the prevention and cause of diabetic complications. There was a debate whether increased levels of blood glucose or exogenous insulin caused diabetic complications. One problem was how to relate glucose levels to diabetic complications since the glucose levels vary considerably in the blood. The detection of glycated haemoglobin (HbA1c) as a marker of the mean glucose level during the previous 2-3 months made comparisons easier. However the first studies failed to show any benefits of improving glycaemic control in reducing the development of diabetic complications. We now understand that this was due to too short study time and small patient materials.

Randomized clinical trials over long periods of time were designed to definitely determine whether intensive treatment, i.e. lowering the blood glucose levels as close as possible to the level of a person without diabetes, could prevent the development of diabetic complications. The Stockholm Diabetes Intervention Study (SDIS) could first present such effects and in 1993 the American study Diabetes Control and Complications Trial (DCCT) confirmed the results in a larger study population (10, 17, 18).

The results of the DCCT was a key step for both insulin treatment and the cause and prevention of diabetic complications. It became apparent that with blood glucose levels as close to normal as possible diabetic complications could efficiently be prevented, but also that the risks for hypoglycaemia, with the ultimate adverse event of coma, increased when lower levels of blood glucose were reached. This led to the modern era of frequent measurements of blood glucose to adjust insulin doses and the development of insulin analogues to improve glycaemic control and avoid hypoglycaemias.

The first insulin analogue introduced was the mealtime insulin, insulin lispro, which had a faster effect than regular insulin. The second insulin analogue introduced was insulin aspart which also was a meal time insulin with a similar effect profile as insulin lispro. Thereafter the basal insulin analogue insulin glargine was introduced and was followed by the basal insulin analogue insulin detemir. Recently also the insulin analogue glulisine which is a meal time insulin has been introduced.

1.4 Diabetes mellitus today

There are different forms of diabetes. The classification used in clinical practice comprises four main types of diabetes (5). Type 1 diabetes is due to destruction of the beta cells and generally leads to absolute deficiency of insulin. Type 2 diabetes is caused by insulin

resistance and a progressive impairment in the insulin secretion. The incidence of type 1 as well as type 2 diabetes is increasing (1). The incidence of type 1 diabetes is largest in Finland with an incidence of 35-40/100,000 which is roughly double the incidence in the United States. The third form includes different subforms of diabetes with other causes such as genetic defects in the beta cell function or insulin effect, diseases of the exocrine pancreas and diabetes induced by chemicals and drugs (5). The fourth form in the classification of diabetes is gestational diabetes defined as diabetes diagnosed during pregnancy. It is sometimes difficult to determine the type of diabetes. A patient who clinically has the typical signs of type 2 diabetes can start with a ketoacidosis. On the other hand a patient with type 1 diabetes can have a late onset and slow progression of disease although antibodies against the beta cells are present.

There are three ways of diagnosing diabetes (5). The test used in the majority of cases in clinical practice is measurement of fasting plasma glucose. If the fasting plasma glucose is repeatedly above 7.0 mmol/l, diabetes is present. The main advantage with measurement of fasting plasma glucose is the simplicity of the test. A second way of diagnosing diabetes is oral glucose tolerance test (OGTT), meaning that 75 g oral glucose load is given during standardised forms. The cut-off for diagnosis is plasma glucose of 11.1 mmol/l or higher 2 hours after the test. The OGTT has a greater sensitivity than fasting plasma glucose but less reproducibility. The test detects 30% of diabetes patients that fasting plasma glucose will not find (19). The third way is when any random sample of plasma glucose is 11.1 mmol/l or higher and typical symptoms of diabetes onset such as polyuria, polydipsia or decrease in weight that cannot be explained by any other cause exist. All three types of tests must be repeated and reproduced during another day if hyperglycaemia does not unequivocally exist.

During recent times increased focus has been on prediabetes, i.e. a stage before the development of diabetes. There is a need to find strategies to halt the development of diabetes. Large trials of both physical activity and drugs have been undertaken to intervene with the condition showing that the onset of diabetes can be delayed (20). Measurements indicating prediabetes are impaired fasting plasma glucose, i.e. plasma glucose higher than normal, but not as high as 7.0 (5). In the guidelines of the American Diabetes Association impaired fasting glucose is defined as plasma glucose 5.6-6.9), whereas the WHO guidelines define it as 6.1-6.9 mmol/L (5, 19). In a corresponding manner impaired glucose tolerance (IGT) can be diagnosed by OGTT, as plasma glucose 7.8-11.0 two hours after the test. The glucose levels in a person with normal glucose metabolism are very stable and hence it can be understood that it is also abnormal to have glucose values in the range of IFG or IGT (Fig. 1). The reason for the difficulties in having a uniform classification of IFG is a lack of evidence in preventing progression to diabetes and adverse events by the use of the lower level of 5.6 mmol/l (19).

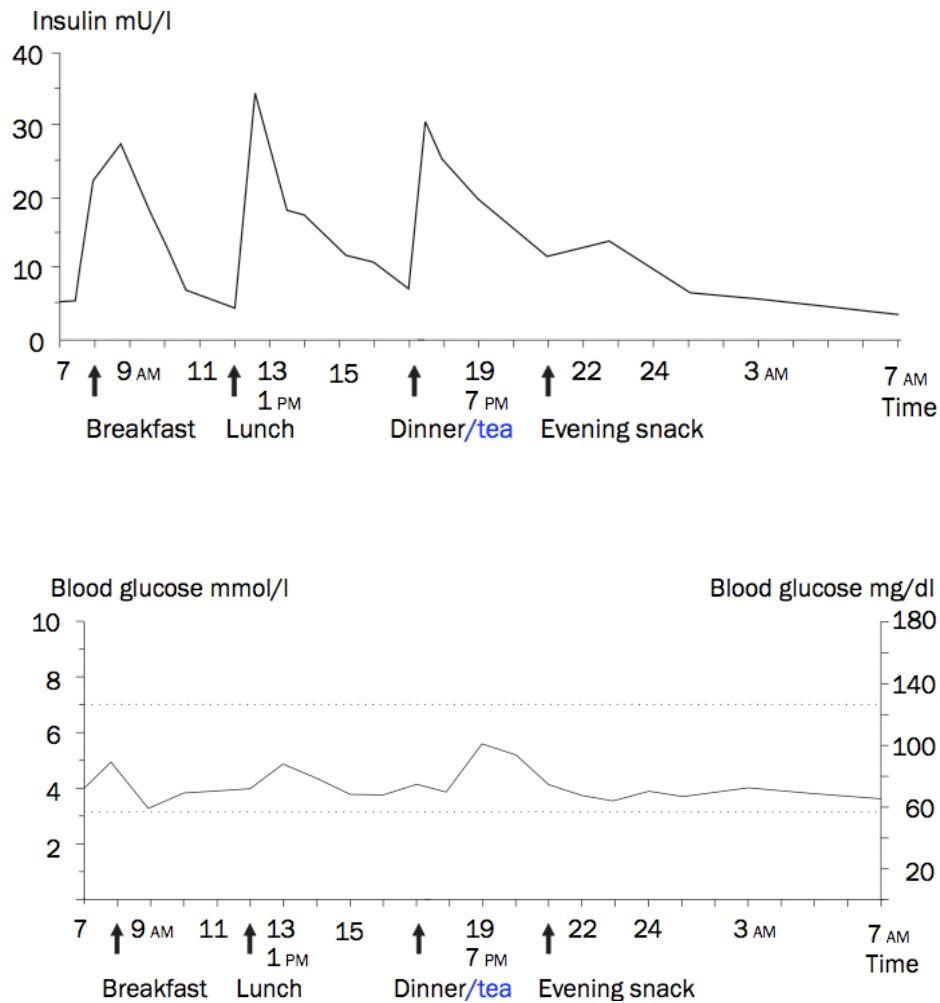


Figure 1: A person without diabetes.

If an individual does not have diabetes, the insulin concentration in the blood will increase rapidly after a meal (21). When the glucose in the food is absorbed from the intestine, and the blood glucose has returned to normal levels, the insulin level will drop back to baseline once again. However, the insulin level will never go right down to zero, as a low level of basal insulin is needed to take account of the glucose coming from the reserve stores in the liver between meals and during the night. The resulting blood glucose level will be very stable in a person without diabetes as this graph illustrates (22). The normal blood glucose level is between about 4 and 7 mmol/l (70-125 mg/dl). *With permission from Diabetes & Metabolism and Betamed (21, 23).*

In type 1 diabetes recommended treatment today is multiple daily insulin injections (MDI) or treatment with continuous subcutaneous insulin infusion (CSII, [5]). Several measurements of blood glucose per day are recommended for adjustments of insulin doses. The basis for this recommendation is the substantial preventive effect on diabetic complications seen in the DCCT by these treatments (5, 10). When MDI is used, NPH insulin as basal insulin or the insulin analogues glargine or detemir are used. As meal time insulin regular insulin or the insulin analogues insulin lispro or aspart have generally been used. Recently also the meal time insulin glulisine has become a treatment option.

In type 2 diabetes metformin is generally the first treatment option due to a preventive effect on diabetic complications and mortality seen in the United Kingdom Prospective Diabetes Study (UKPDS, [5, 24]). It is also recommended that insulin treatment should be initiated early to keep glucose levels on a low level. Besides these general recommendations there are many options in type 2 diabetes both with different oral antidiabetic agents and subcutaneous injections affecting the incretine system (25). Mix-insulins which are a mix of medium-acting insulin and insulin lispro or aspart are also a common treatment option besides the insulins used in type 1 diabetes.

Chapter 2 Effects of modern insulin analogues

The two most commonly used basal insulins today are the NPH insulin introduced many decades ago and the modern insulin analogue glargine. For meal time insulins the insulin analogues lispro and aspart as well as regular insulin are most commonly used. Insulin lispro and aspart have in principle identical effect profiles and are usually considered as equivalent treatment options. In this thesis the effects of insulin glargine and NPH insulin on HbA1c have been compared as well as those of insulin lispro and regular insulin.

Insulin glargine has a more physiologic effect profile than NPH insulin in the sense that it more closely mimics the natural level of basal insulin in people without diabetes (26, 27). In comparison with NPH insulin, insulin glargine has a longer time of insulin absorption and lower peak (Fig. 2). The more flat effect profile of insulin glargine could possibly be an advantage in stabilising blood glucose levels. The absence of a pronounced peak could also possibly help to avoid nocturnal hypoglycaemias. Another potential advantage has been longer effect duration of 20-24 hours and the possibility of only taking insulin glargine once daily to cover the basal insulin need. In insulin glargine lysine has been replaced by asparagine at the position A21 of the insulin molecule as well as an addition of two arginine molecules on B30 (13). It leads to a shift in the isoelectric point and a molecule which is less soluble at the injection site. Insulin glargine dissociates in the subcutaneous tissue to a depot and is then slowly released.

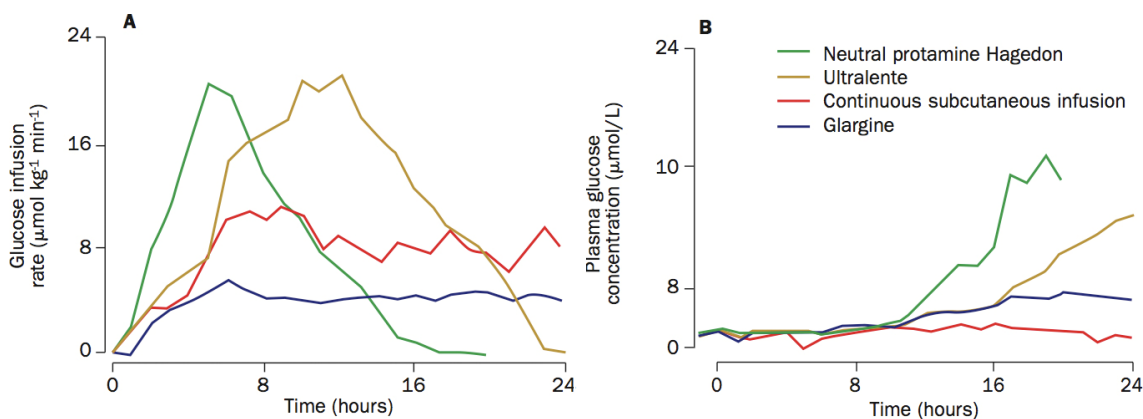


Figure 2. Time-action characteristics of the protracted-acting insulins neutral protamine Hagedorn, ultralente, and glargine, and continuous subcutaneous infusion with insulin lispro. Insulins were given by bolus subcutaneous injection in the medial aspect of the thigh at 0.3 U/kg, continuous subcutaneous infusion at 0.3 U/kg at time 0. (Left) glucose infusion rates needed to maintain plasma glucose at 7.2 mmol/L. (Right) corresponding plasma glucose concentrations. Intravenous glucose was withdrawn when plasma glucose exceeded 7.5 mmol/L. Adapted with permission from Lepore et al, 2000. *With permission from the Lancet* (28).

The effect profile of insulin lispro compared to regular insulin mimics more the fast insulin response at meals seen in people without diabetes (Fig. 1). Injections of insulin lispro result in

higher maximal concentration and is reached in a shorter period of time than regular insulin (Fig. 3). Possible advantages with insulin lispro are lower risk for hypoglycaemias and better glycaemic control due to the more physiologic insulin coverage. Insulin lispro is taken in close connection to the meals which might also facilitate adjustments of insulin doses. The rapid effect of insulin lispro is due to a faster dissociation to monomers in subcutaneous tissue (13). The property of less association was obtained by changing the places of the amino acids lysine on B29 and proline on B28. These shifts of amino acids were made with the inspiration of IGF-1 which does not have the tendency to self-associate.

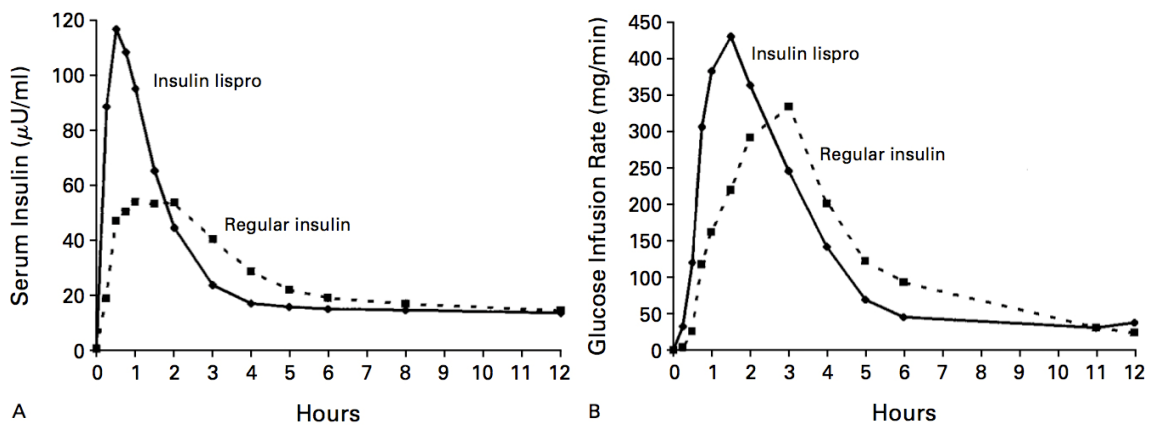


Figure 3. Effects of Subcutaneous Administration of Insulin Lispro and Regular Insulin on Serum Insulin Concentrations (Panel A) and the Rate of Glucose Infusion Necessary to Maintain Normoglycemia (Panel B) in 10 Normal Subjects. To convert values for insulin to picomoles per liter, multiply by 6.0; to convert values for the glucose infusion rate to millimoles per minute, multiply by 0.005551. Data were adapted from Howey et al. *With permission from the New England Journal of Medicine* (29).

2.1 Insulin glargine in randomized clinical trials

In randomized clinical trials (RCTs) of insulin glargine and NPH insulin the designs have been similar in several aspects:

- 1) Patients treated with NPH insulin are included and randomized to insulin glargine or continuing with NPH insulin.
- 2) Studies have not been blinded.
- 3) NPH insulin and insulin glargine are titrated by certain goals of FPG. The same goals have been used for both groups.

The studies mainly differ with regard to

- 1) size and duration
- 2) definitions and the way of registering of hypoglycaemias
- 3) the level of FPG to which titrations are made.

Sometimes the point of time when insulin glargine and NPH insulin are injected have also differed. In studies of type 1 diabetes differences also exist depending on whether titrations schedules for meal time insulins have been used. In these studies the types of meal time insulins have also differed. In type 2 diabetes different oral antidiabetic agents have been used in combination with insulin therapy. Sometimes patients with multiple daily injections in type 2 diabetes have also been studied.

Type 1 diabetes

Early studies of insulin glargine were made with fasting plasma glucose (FPG) as the primary endpoint. These studies were short, only 4 weeks, and during the first three weeks titrations of insulin doses were made and during the fourth week FPG was measured daily. Hence, no relevant evaluation of HbA1c could be made (30, 31).

Raskin compared over 16 weeks insulin glargine and NPH insulin for patients treated with insulin lispro as mealtime insulin (32). NPH insulin was given as one dose or several doses per day. The study comprised 619 patients. All patients continued with the same doses of insulin lispro as previously, i.e. no special optimization scheme was made for the mealtime insulin. NPH insulin and insulin glargine were titrated so that fasting plasma glucose reached 4.7-6.7 mmol/l. In the patient material 75% had NPH insulin two or more times per day. Hypoglycaemias were divided into symptomatic, nocturnal and severe. The incidence for hypoglycaemias was reported but it is unclear how the different types of hypoglycaemias were strictly defined. In this study no significant differences in the level of HbA1c or the frequency of hypoglycaemias were found between the groups. However, the level of FPG was lower in patients treated with insulin glargine.

Rossetti compared NPH insulin given 4 times per day with insulin glargine over 3 months (33). Both groups had lispro as meal time insulin. There were 51 patients included who were randomized to NPH insulin, insulin glargine at dinner or insulin glargine in the evening. Measurements and adjustments of insulin doses were made frequently. Fasting and preprandial blood glucose levels were measured daily, postprandial values every second day and values during the night at 03.00 twice a week. Goals for blood glucose levels were fasting, preprandial and at bedtime 6.4-7.2. The goals 2 hours postprandially were 8.0-9.2. If fasting blood glucose differed from 6.0-7.8, the insulin glargine dose was changed. NPH insulin was adjusted to each meal from preprandial blood glucose levels. Insulin lispro was adjusted daily on the basis of preprandial blood glucose levels, postprandial values during the previous days as well as from the composition and size of meals and physical activity. Hypoglycaemia was defined as any value below 4.0 mmol/l and severe if external help was needed. Patients treated with insulin glargine decreased significantly more in HbA1c, had fewer hypoglycaemias and lower levels of fasting blood glucose.

The same research group later made a longer and larger study over 1 year of 121 type 1 diabetes patients (34). The study design was very similar where patients with NPH insulin 4 times per day and insulin lispro as mealtime insulin were randomised to insulin glargine or continuation with the previous regime. Frequent measurements of blood glucose and titration schedules of insulins were made in a similar manner. After 4 months HbA1c in the insulin glargine group had dropped from 7.1% to 6.7% whereas it remained on the same level for patients treated with NPH insulin. After one year HbA1c was still on the same level for patients with NPH insulin, whereas it decreased from 7.1 to 6.6 for patients treated with insulin glargine. Mild hypoglycaemia defined as glucose values below 4.0 mmol/l was more frequent in those with NPH insulin (13.2%/patient/month) than in those with insulin glargine (7.2%/patient/month). The level of fasting glucose was lower for patients treated with insulin glargine.

Ratner compared NPH insulin and insulin glargine in 534 patients with type 1 diabetes during 28 weeks (35). Meal time insulin was regular insulin. The doses of NPH insulin and insulin glargine were titrated to reach fasting blood glucose of 4.4-6.7 mmol/l. Both groups received regular insulin 30 minutes before meals, but no optimization schedule was present. At eight visits fasting glucose was measured. Hypoglycaemias were divided into severe and nocturnal. Fewer hypoglycaemias were reported with insulin glargine. At endpoint no difference was seen in HbA1c or fasting blood glucose between the groups.

Hence, in RCTs of type 1 diabetes the effects on HbA1c have shown divergent results but in the majority of studies there have been fewer hypoglycaemias. A recent meta-analysis showed a decrease in HbA1c of 0.11 percentage units with insulin glargine (36).

Type 2 diabetes

Riddle found no effect of insulin glargine on HbA1c or FPG in a study of 756 type 2 diabetes patients (37). However, patients treated with insulin glargine had significantly fewer nocturnal hypoglycaemias (26%) than patients with NPH insulin (32%). The patients had 1 or 2 oral antidiabetics and HbA1c above 7.0% before randomization. Titrations were made to reach fasting glucose <5.6 mmol/l. Massi Benedetti did not either find any effect on HbA1c or hypoglycaemias in a similar study of 570 patients over 52 weeks (38). Insulin glargine and NPH insulin were given once daily at bedtime and the oral antidiabetics used before the study

were continued during the study. However, nocturnal hypoglycaemias were also here less common in patients treated with insulin glargine. Also in a similar study Yki-Järvinen found no effect on HbA1c in 426 patients with poor glycaemic control over 1 year (39). However, fewer nocturnal hypoglycaemias were seen with insulin glargine (9.9%) compared with NPH insulin (24.0%). Insulin glargine and NPH insulin were taken at bedtime and titrations made to fasting glucose <6.7 mmol/l. Treatment with oral antidiabetics was continued in the same way as previously. Yki-Järvinen also later examined 110 patients over 36 weeks with poor glycaemic control receiving metformin and randomised to either insulin glargine or NPH insulin (40). No differences in HbA1c or hypoglycaemias were seen.

Fritsche made a study of 695 type 2 diabetes patients over 24 weeks (41). Randomization was made to morning insulin glargine, glargine at bedtime or NPH insulin at bedtime. Titrations were made to fasting glucose <5.6 mmol/l and all groups received glimepiride 3 mg. Patients receiving morning insulin glargine decreased significantly more than the two other groups in HbA1c. HbA1c decreased by 1.24 percentage units with morning insulin glargine, 0.96 with glargine at bedtime and 0.84 with NPH insulin. Fasting glucose was improved in the same range in all groups. Nocturnal hypoglycaemias were less common with morning and bedtime insulin glargine than with NPH insulin. Eliaschewitz and Pan have also studied insulin glargine in combination with glimepiride in two separate studies in Latin America and Asia respectively (42, 43). Eliaschewitz found no effect on HbA1c but fewer nocturnal hypoglycaemias with insulin glargine in 481 patients over 24 weeks (42). Pan showed on the other hand in a similar study over 24 weeks of 443 patients a beneficial effect with insulin glargine on both HbA1c and hypoglycaemias (43).

Rosenstock studied 518 patients with type 2 diabetes during 28 weeks (44). The patients had NPH insulin in one or several doses before randomization without oral antidiabetics. Regular insulin was taken to the meals. Patients were randomized to NPH insulin or insulin glargine and titrations made to reach morning fasting glucose of 4.0-7.8 mmol/l. Bedtime insulin was reduced if there was a nocturnal hypoglycaemia. Preprandial goal of blood glucose was 4.0-7.8 mmol/l and at bedtime 6.7-10.0 mmol/l. Hypoglycaemia was defined as symptomatic and with blood glucose level below 2.8 mmol/l. No difference was found in the total amount of hypoglycaemias between the groups but the numbers of nocturnal hypoglycaemias were fewer for the glargine group. No difference was found in fasting glucose or effect on the HbA1c level.

Hence the majority of RCTs of insulin glargine in type 2 diabetes have not found any superior effect on HbA1c with insulin glargine but significant effects on hypoglycaemias (Table 2). A recent meta-analysis on the topic showed similarly no superior effect on HbA1c but fewer hypoglycaemias (45).

Table 2: Effects on HbA1c and hypoglycemias in RCT:s of type 2 diabetes

Study	Year	Effect on HbA1c	Effect on hypoglycemia
Yki-Järvinen	2000	No	Yes
Rosenstock	2001	No	Yes
Fritsche	2003	Yes	Yes
Massi Benedetti	2003	No	Yes
Riddle	2003	No	Yes
Eliaschewitz	2006	No	Yes
Pan	2007	Yes	Yes
Yki-Järvinen	2006	No	No

2.2 Studies of insulin glargine in clinical practice

In an uncontrolled retrospective study of 49 patients HbA1c decreased significantly by 1.1 percentage units when switching from NPH insulin to insulin glargine (46). The frequency of severe hypoglycaemias was also significantly reduced. In the study 93.1% had type 1 diabetes. In another retrospective uncontrolled study of 43 patients HbA1c increased non-significantly by 0.1 percentage unit when changing from NPH insulin to insulin glargine (47). There was no difference in the frequency or severity of hypoglycaemic episodes. In a study with similar design of 136 type 1 diabetes patients HbA1c had decreased 0.4 percentage units 3 months after the change from NPH insulin to insulin glargine (48). At six months the decrease was still significant of 0.2 percentage units and also after a year of 0.3 percentage units. In another uncontrolled retrospective study of 83 patients with type 1 diabetes HbA1c decreased over 1 year non-significantly by 0.1 percentage unit when changing from NPH insulin to insulin glargine (49). The frequency of severe hypoglycaemia with unconsciousness decreased after the change to insulin glargine.

The effect on glycaemic control by the use of an educational program together with the introduction of insulin glargine has also been studied (50). In this retrospective analysis of patients with type 1 diabetes there were 54 patients changing from NPH insulin to insulin glargine. Glargine was given at bedtime. HbA1c decreased significantly by 0.14 percentage units. No severe events of hypoglycaemia occurred.

Hence, studies in clinical practice of insulin glargine and NPH insulin have shown divergent results concerning effects on glycaemic control and frequency of hypoglycaemias.

2.3 Rapid-acting insulin analogues

The rapid-acting insulin analogues insulin aspart and lispro were compared with regular insulin in a meta-analysis (51). Twenty randomized clinical trials of type 1 diabetes and four of type 2 diabetes were included. On average patients with type 1 diabetes receiving rapid-acting insulin analogues reached 0.12 percentage units (0.07-0.17) lower HbA1c than patients

with regular insulin. In type 2 diabetes there was no difference in HbA1c between patients treated with rapid-acting insulin analogues and regular insulin. None of the included studies of type 2 diabetes showed any difference in HbA1c. Concerning hypoglycaemias there was no difference in the frequency between the treatments for either type 1 or type 2 diabetes.

Lunt studied as a complement to clinical trials the effect of insulin lispro on HbA1c in clinical practice (52). The study was prospective and included patients with regular insulin before the main meals and NPH insulin as basal insulin. The patients were followed for at least one year. There were 190 patients who changed treatment to insulin lispro and 94 who continued with regular insulin. No change in HbA1c was found. Those with high HbA1c decreased most in HbA1c when changing to insulin lispro. No such effect was seen in the control group and the authors therefore believe the better effect of lispro at high HbA1c levels was not due to regression to the mean. Patients treated with insulin lispro had fewer hypoglycaemias than patients with regular insulin.

Stocks examined the effect of changing from conventional insulins to insulin lispro in type 1 diabetes patients in clinical practice with poor glycaemic control (53). There were 150 patients included of whom 125 completed the study. There was no control group continuing with conventional therapy. HbA1c decreased significantly and the reduction was most pronounced in patients with moderately increased HbA1c 8%-9%. The HbA1c level was not significantly changed for those with HbA1c below 8.0%. Both the frequency of hypoglycaemias during the day and nocturnal hypoglycaemias decreased when the treatment was changed to lispro.

Chatterjee examined prospectively in an uncontrolled study patients starting with insulin lispro (54). Patients with the following conditions were included: 1) problems to wait between injections and meals 2) large postprandial hyperglycaemias 3) late postprandial hypoglycaemia 4) nocturnal hypoglycaemia. In total 221 diabetes patients were included of whom 198 had type 1 diabetes and 23 patients had type 2 diabetes. After 6 months 211 patients were followed-up and HbA1c had then decreased from 9.11% to 8.56%. At 1 year 177 patients were followed up and HbA1c was then 8.78%. There was no difference in the frequency of severe hypoglycemia before and after the change to insulin lispro. However, scores of self-assessed hypoglycaemias decreased significantly.

Chapter 3 Definitions and clinical course of diabetic complications

3.1 Retinopathy

Diabetic retinopathy develops in the majority of patients with diabetes (55). In type 2 diabetes there were as many as one third of all patients with retinopathy at the time for diagnosis in the UKPDS (56). In more recent estimates of the prevalence of retinopathy at diagnosis in type 2 diabetes approximately 20% have retinopathy and in 20 years 60% have developed retinopathy (55). A recent follow-up of the Wisconsin Epidemiology Study of Diabetic Retinopathy (WESDR) estimated the cumulative progression and regression of retinopathy in type 1 diabetes over 25 years (57). There were 83% of the 955 studied patients that progressed in retinopathy, 42% progressed to proliferative retinopathy and 18% experienced a regression. In economically developed countries diabetic retinopathy is the most common cause of visual disability and legal blindness in the age group 20-74 years of age (58, 59). Risk factors for development of diabetic retinopathy are impaired glucose control, long diabetes duration and hypertension (56, 60-63). Male gender was also a risk factor in the WESDR and surprisingly smoking had in the UKPDS a protective effect of retinopathy (56, 57). Good glycaemic control and tight blood pressure control have proven preventive of diabetic retinopathy in clinical trials (10, 56). Good glycaemic control and photocoagulation are the corner stones in the prevention and treatment of retinopathy (58). In advanced stages of retinopathy photocoagulation can reduce the progression to blindness by more than 50%.

The two most common causes of visual loss in diabetes are macula oedema and retinal neovascularisation (59). The reason for visual loss is different in type 1 and type 2 diabetes. In type 1 diabetes visual loss is mainly due to the forming of new vessels in the fundus of the eye and the development of proliferative retinopathy. In type 2 diabetes visual loss is mainly caused by macula oedema. Proliferative retinopathy is relatively speaking uncommon in type 2 diabetes. In diabetes patients over 40 years of age 40% have retinopathy and in 20% of them the vision is threatened (55). In the age group over 50 years the risk of visual impairment is twice that for a person without diabetes.

Microaneurysms/bleedings, changes in the blood retina barrier, capillary closure and changes in the neuronal and glial cells of the retina are characteristic of the early phase of diabetic retinopathy (59). Retinopathy and other microvascular complications of diabetes develop due to chronic hyperglycaemia which leads to injuries on the blood vessels (55). Increased permeability of the vessels, vascular leakage and vascular oedema appear. The tonus of the vessels increases due to hyperglycaemia which leads to increased blood pressure. There is also an accumulation of the extracellular matrix resulting in a thickening of the basement membrane which can lead to occlusion and ischemia. The ischemia can cause visual impairment due to hypoxaemia and death of nerve cells in the retina. To compensate for the hemodynamic alterations new blood vessels develop but these vessels are fragile and lead to risks of bleeding which instead can impair the vision.

There have been several ways of classifying retinal lesions due to diabetes (55). Classification is important for prognosis and overall estimations of the seriousness of the retinal lesions. An

adequate classification of the seriousness is also important when estimating how different risk factors influence the severity of retinopathy as well as when new treatments are evaluated. If a classification does not mimic the true severity of injuries in the eyes, it would have been difficult to assess whether e.g. intensive blood glucose control is important in preventing retinopathy. A very accurate classification was thus urgently needed when evaluating intensive blood glucose control e.g. in the DCCT and UKPDS. In fact development and progression of retinopathy were the primary endpoints in the DCCT (10). The first classification was the Airlie House Classification. It was developed in 1968 at a meeting at Airlie House in Warrington, VA (59). The purpose was to develop a classification for different grades of serious lesions of retinopathy that could be detected by ophthalmoscopy or photographs of the fundus.

The Early Treatment Diabetic Retinopathy Study (ETDRS) Research Group developed in 1991 a modification of the Airlie House Classification (59, 64). The ETDRS scale is the one used in the hallmark studies DCCT and UKPDS (10, 56). The ETDRS was developed to classify different grades of seriousness of retinopathy due to the probability of progressing to proliferative retinopathy (59). Small modifications have been made of the ETDRS scale and in the most recent studies generally the so-called final version of the ETDRS scale has been used. The final version of the ETDRS scale comprises 23 steps of retinopathy grading where 3 steps of worsening is considered as a significant progression of retinopathy. Since retinopathy sometimes can be ameliorated and go back to a less advanced stage generally sustained retinopathy in the DCCT was classified as three steps progression that is sustained a half year later. In Table 3 a shortened version of the ETDRS scale is presented.

Table 3: Grading system

Level	Severity	Definition
10	DR absent	All diabetic retinopathy features absent
20	MA only	Microaneurysm(s) only, other lesions absent
35	Mild NPDR	MA plus haemorrhage(s) and/or hard exudates and/or cotton wool spots
43	Moderate NPDR	Lesions as above + either extensive or severe HMA or IRMA present
47	Moderately severe NPDR	Lesions of 35 + either extensive or severe HMA with IRMA, or venous beading
53	Severe NPDR	Extensive and severe HMA, IRMA, and/or venous beading
61, 65, 71, 75, 81	Proliferative DR	NVD and/or NVE without or with complications

where DR = diabetic retinopathy, NPDR = non-proliferative diabetic retinopathy, MA = microaneurysm, HMA = haemorrhages and microaneurysms, HE = hard exudates, CWS = cotton wool spots, IRMA = intraretinal microvascular abnormalities, NVD = new vessels on the disc, NVE = new vessels elsewhere

With permission from Diabetologia (56).

3.2 Nephropathy

Diabetes is the leading cause of end stage renal disease (ESRD) and constitutes approximately 40% of all patients needing renal replacement therapy (65). Type 2 diabetes is the largest and fastest growing disease which is in need of renal replacement therapy. Besides the high risk of ESRD due to diabetic nephropathy there is also a high risk for cardiovascular morbidity and mortality. In recent years much focus has been put on finding the causes and possible preventions to avoid impairment of the renal function in diabetes patients. Greater emphasis has been on identification of risk factors and interventions at a very early stage of disease to avoid progression to ESRD. Good glycaemic control and blood pressure control reduce cardiovascular (CV) events and development of nephropathy. In more advanced stages of nephropathy tight blood pressure control has a greater impact to prevent kidney progression than good glycaemic control.

Diabetic nephropathy is characterised by changes in the glomerulus filtration rate, expansion of extracellular matrix in the mesangial part, i.e. in the central part of the glomerulus, glomerular capillary crowding and overt renal occlusion leading to kidney failure (55). The clinical syndrome diabetic nephropathy is characterised by persistent albuminuria (>300 mg/24h or 200 μ g/min), early increase in arterial blood pressure and irreversible decline in GFR (66). The first sign of diabetic nephropathy is usually albuminuria and the first symptom peripheral oedema. The grade of decline in GFR in the natural history of diabetic nephropathy has been found to be highly variable (2-20 ml/min/year) with a mean of 12 ml/min/year.

Microalbuminuria is defined as persistent urine albumin excretion in the range of 30-300 mg/24 hours or 20-200 μ g/min (5, 66). It is an important marker of increased risk for nephropathy, ESRD as well as cardiovascular morbidity (5, 65). It has therefore been possible to identify patients with high risk at an early stage of disease. Patients progressing from microalbuminuria to macroalbuminuria have a large risk of progressing to ESRD (5). In general the albumin/creatinine ratio is measured in clinical practice to estimate micro and macroalbuminuria. The reason is that 24 hour collections of urine albumin mean much more work but add little in predictive ability and accuracy. The cut-off for microalbuminuria is then 30–299 μ g/mg creatinine and for macroalbuminuria ≥ 300 μ g/mg creatinine. Measurement of only albumin in a spot urine without simultaneous measurement of urine creatinine has been used in clinical practice and studies but a greater risk follows for false negative and positive results. The measured level of albuminuria is dependent on hydration and other factors. Exercise within 24 hours, fever, infections, CHF, marked hyperglycemia and marked hypertension can possibly increase the level of albuminuria. Thus, two of three positive tests of microalbuminuria within half a year is recommended for diagnosis (5).

Creatinine is also used in clinical practice and studies to estimate GFR and predict the grade of chronic kidney disease (5, 67). Renal failure due to diabetes was e.g. in the UKPDS defined as creatinine above 250 or need of dialysis and that no other acute disease can have caused the renal impairment. Another reason why creatinine is measured is that studies have found decreased GFR in several adult diabetes patients although normoalbuminuria is present (5). Hence, in some patients measurements of albuminuria could miss detecting an impairment in renal function. Below, the definitions of microalbuminuria used in some of the landmark studies of diabetes and nephropathy are presented. The number of positive tests for diagnosis of nephropathy has varied somewhat in studies where only one positive test has sometimes been used as endpoint whereas in other cases two of three positive tests were used (10, 67-70).

Table 4: Different definitions used of microalbuminuria in 5 clinical trials of nephropathy and in clinical guidelines

Study	Dipstick	A/C-ratio	24h U-albumin	microg/min
HOPE		> 2,0 in one sample		
UKPDS	> 50 mg in one sample			
DCCT			40 mg/24 h in one sample	
ADA-guidelines*			30-299 mg/24h in 2 of 3 samples	
Eurodiab				20-200microg/min in one sample
Oslo study			Repeatedly >27 mg/24h	

*ADA=American Diabetes Association

3.3 Macrovascular complications

The major cause of morbidity and mortality in people with diabetes is cardiovascular disease (CVD [5]). CVD also constitutes the largest part of direct and indirect costs associated with diabetes. The risk for mortality in diabetes is approximately doubled in comparison with people without diabetes in the same age group (71-73). Macrovascular complications are generally manifested as myocardial infarction or stroke. Approximately two thirds of all deaths in patients with diabetes are caused by cardiac disease or stroke (73). The death rate from heart disease and the risk of stroke in adults with diabetes is 2-4 times higher than in people without diabetes. Macrovascular complications in diabetes also have a more severe course with higher prevalence of affection of several coronary vessels and more elongated atheromas (74). An exceptionally high risk is present in patients with type 2 diabetes who have had a first CV event (75-78).

Since a long time ago it has been known that the risks of CVD are increased for diabetes patients. The classic concept has been that macrovascular disease is a diabetes specific complication, but it has also been discussed whether both type 2 diabetes and CVD stem from the same etiologic causes in form of genetics and environment (79). There are e.g. many risk factors such as hypertension and hyperlipidemia but also factors associated with insulin resistance that are the same for both CVD and type 2 diabetes. Hence the possibility of different origins of macrovascular complications can make the estimations of the individual importance of different risk factors complex. Accurate estimations of the importance of diabetes and the glucose control for the development of CVD in type 2 diabetes could e.g. be difficult if both conditions in part stem from the same origin.

Chapter 4 The relation of HbA1c and diabetic complications

Some of the most frequently asked questions in research concerning the associations between glycaemic control and diabetic complications are:

1. Is the glycaemic control at all important for the development of diabetic complications?
2. How important is the glycaemic control for the development of diabetic complications, i.e. if the glucose levels are reduced to a certain extent how many complications can be saved?
3. Is there a threshold of glycaemic control below which the risks for diabetic complications disappear?
4. Does improvement of the glycaemic control only affect the initial stages of diabetic complications or can it also be preventive when diabetic complications have begun to appear?
5. Assume that the glucose levels have been lower for a period and been beneficial in preventing diabetic complications. Will this salutary effect disappear if the glucose levels later increase?

When evaluating glycaemic control in clinical practice the laboratory marker glycated haemoglobin (HbA1c) is the golden standard (80). HbA1c describes the blood glucose level during the last 2-3 months and is the general measure when goals of glycaemic control in guidelines are set. It is also the marker used when evaluating new pharmacological treatments and when assessing the importance of glycaemic control in the development of diabetic complications.

The glycation of haemoglobin as an estimate of the blood glucose level in diabetes patients was first presented in 1976 (81). Epidemiologic studies such as the Epidemiologic Study of Diabetic Retinopathy (WESDR) could later show an association between higher levels of HbA1c and the development of retinopathy (82, 83).

During the same period a couple of clinical trials were presented which however showed no salutary effect on retinopathy when HbA1c was lowered. One of these studies was a multicenter randomized clinical trial of 70 diabetes patients over 8 months with continuous subcutaneous insulin infusion (CSII) with the aim of as close to normal blood glucose as possible (84). Patients with intensive treatment had no effect in preventing retinopathy but possibly a somewhat deleterious effect. The authors concluded that longer studies must be designed and that it was possible to obtain intensive control in a randomised setting in a feasible manner. The second study was a similar study of 30 type 1 diabetes patients over two years neither showing any conclusive beneficial effects on retinopathy (85).

In 1989 the randomized Stockholm Diabetes Intervention Study (SDIS) of 96 type 1 diabetes patients was the first trial showing positive preventive effects on the development of diabetic complications, namely less nephropathy with intensive therapy (17). In July 1993 the SDIS study presented long-term beneficial effects of intensive treatment on retinopathy, nephropathy and neuropathy (18). During the same period several epidemiologic studies

showed relations between higher HbA1c levels and retinopathy, albumin excretion rate, nephropathy, ulcers and amputations (86-89).

In September 1993 the DCCT results were presented which confirmed the beneficial effects of intensive glycaemic control observed in the SDIS on retinopathy, neuropathy and nephropathy in 1,441 type 1 diabetes patients over on average 6.5 years (10). The fact that the DCCT was both a large study and randomised intervention trial made it the landmark study of type 1 diabetes changing the era to intensive treatment as the golden standard for type 1 diabetes patients. Since the DCCT was the only study that was large, included frequent measurements of risk factors such as HbA1c and recordings of outcomes many later reports of relations of glycaemia to complications for guidelines in type 1 diabetes have stemmed from the DCCT (5).

In 1998 the corresponding long and large clinical trial of type 2 diabetes patients was presented, namely the UKPDS (90). In the DCCT it had not been possible to study the effect on cardiovascular disease (CVD) due to the young age of the participants. In the UKPDS, as in the DCCT, fewer microvascular complications with intensive therapy were seen. However, no significant beneficial effect in preventing CVD was found although the effect on myocardial infarction was of borderline significance. Hence, the importance of lowering HbA1c in preventing CVD has been intensively debated and further studied after the UKPDS.

4.1 Retinopathy

The DCCT study has been the basis together with the UKPDS when examining associations between glycaemia and retinopathy (10, 90). In the DCCT most analyses have been on retinopathy which also was the primary endpoint in the DCCT. Retinopathy was also most frequently recorded, namely every half year, compared to microalbuminuria which was evaluated yearly and neuropathy which was evaluated at start and after 5 years. Most outcomes of retinopathy also appeared since it is generally first detected of the diabetic complications. The fact of frequent recordings and many outcomes increases the power and makes detailed analyses of associations between HbA1c and retinopathy possible.

When the first analyses of the DCCT were presented it was established that intensive blood glucose control is of importance in preventing retinopathy, microalbuminuria and neuropathy (10). The difference in HbA1c levels between the intensive and conventional groups was 1.9 percentage units (9.1% vs. 7.2%) and the study length on average 6.5 years. For patients in the primary cohort, i.e. patients without any signs of retinopathy, intensive glycaemic control reduced adjusted mean risk for development of retinopathy by 76% (CI 62-85) in comparison with conventional treatment. In patients with mild to moderate retinopathy intensive treatment reduced progression of retinopathy by 54% (CI 39-66) and reduced the worsening to proliferative or severe non-proliferative retinopathy by 47% (CI 14-67). Hence, there were clear differences in both the development and progression of retinopathy for intensive and conventional treatments. It was also shown that the risks of hypoglycemia increased with lower levels of HbA1c.

Epidemiologic analyses of the DCCT were later performed to assess the importance of glycaemic control and other risk factors for progression of retinopathy (60). In each of the treatment groups mean HbA1c during the trial was the strongest predictor for progression of

retinopathy. The risk gradient was in the same range for both groups. A reduction of HbA1c by 10% (e.g. 8% vs. 7.2%) was associated with 43% lower risk in the intensive group and 45% lower risk in the conventional group. The strongest baseline predictors were prestudy HbA1c and diabetes duration.

In clinical practice it is surprising that some patients with poor glycaemic control avoid diabetic complications and that patients with good glycaemic control sometimes develop complications. The 20% of patients with the best and worst glycaemic control in the DCCT were separately analysed concerning development and progression of retinopathy (61). Among those 153 patients with good glycaemic control defined as mean HbA1c below 6.88%, retinopathy still developed in 9.8%. On the other hand there were 43% who avoided retinopathy although poor glycaemic control existed (HbA1c \geq 9.49%). HbA1c at baseline and BMI were significant predictors besides duration and the average glycaemic control which had the strongest influence on retinopathy. A similar focus was made in another analysis of the DCCT, namely if there was any threshold of HbA1c for the development of retinopathy (91). However, no such threshold was found, but instead there was a continuous decrease in the risk of retinopathy with lower HbA1c levels. A recent analysis has also shown that the type of treatment was of low importance in the DCCT but instead the mean HbA1c level was strongly related to diabetic complications (92).

The Epidemiology of Diabetes Interventions and Complications study (EDIC) which is the follow-up of the DCCT examined whether the salutary effects in the DCCT remained after the end of the trial (93). Shortly after the end of the DCCT both previous randomized groups had in principle the same HbA1c levels. At the four-year follow-up of the EDIC, patients with previous intensive therapy still had less degree of retinopathy. In fact patients with previous intensive therapy also developed fewer new lesions of retinopathy after the DCCT than those with previous conventional therapy.

In the UKPDS 3,867 newly diagnosed type 2 diabetes patients with the median age 54 years were included (90). Randomization was made to intensive treatment with sulfonylurea or insulin or to conventional treatment with a diet. Intensive treatment aimed at an FPG below 6 mmol/l and conventional treatment to as good fasting blood glucose as possible and drugs were added if symptoms occurred or FPG exceeded 15 mmol/l. During 10 years HbA1c in the intensive group was 7.0% and 7.9% in the conventional group. Intensive treatment reduced retinopathy by 21%. In an epidemiologic study 1,919 of the participants in the UKPDS who all had complete data and retinal photos at entry and 6 years later were studied (56). The purpose was to determine risk factors of diabetic retinopathy over 6 years from diagnosis. In total 37% of the patients had retinopathy already at diagnosis illustrating that diabetes for many participants had started many years before it was detected. The development of retinopathy was strongly related to baseline HbA1c, mean HbA1c during the 6 years, higher blood pressure and non-smoking. Progression of retinopathy in those who already had retinopathy was associated with male gender, baseline HbA1c, mean HbA1c during the 6 years and non-smoking.

4.2 Nephropathy

In the DCCT intensive treatment reduced the development of microalbuminuria (≥ 40 mg/24 h) by 39% (95% CI 21-52) and macroalbuminuria (≥ 300 mg/24 h) by 54% (95% CI 19-74) [10]. Beneficial effects with intensive treatment were also seen on the development of microalbuminuria and proteinuria in the UKPDS (90). The relative risk reduction in the UKPDS for development of microalbuminuria or proteinuria was 30%. In a study of 110 Japanese patients with type 2 diabetes, patients randomised to intensive treatment reduced microalbuminuria by 62% during 6 years of follow-up (94). Several epidemiologic studies have also illustrated a relation between higher levels of HbA1c and increased risk for development of micro- and macroalbuminuria (68, 95-100).

The importance of glycaemic control in preventing progression from microalbuminuria to overt nephropathy has been less well studied. In the DCCT there were only 10 patients who progressed from microalbuminuria at baseline to macroalbuminuria and hence the power for analyses was low (101). From these 10 patients no effect of intensive treatment could be shown. However, another study of 36 type 1 diabetes patients with microalbuminuria randomising patients to CSII or conventional treatment showed a preventive effect on progression from microalbuminuria to macroalbuminuria (102). The difference in HbA1c was 1.4 percentage units (7.2% vs. 8.6%) over the study period of 2 years. In the UKPDS of type 2 diabetes the progression rate from microalbuminuria to proteinuria was not reported (90). However, there was a significant reduction in progression of microalbuminuria to proteinuria with intensive therapy in the Kumamoto study of type 2 diabetes (94). There are also epidemiologic studies reporting lower risks for progression from micro to macroalbuminuria with lower HbA1c levels (69, 103).

Regression and remission of microalbuminuria defined as 50% reduction in the urine albumin excretion rate and later presence of normoalbuminuria have also been associated with lower HbA1c levels (104). It has also been shown that the lower the HbA1c, the lower risk for nephropathy, i.e. no threshold for beneficial effects exists (67, 91). Besides, it has been evaluated whether intensive therapy has a sustained effect on renal outcomes. In the EDIC follow-up 8 years after the end of the DCCT renal outcomes were evaluated for the former intensive and conventional groups (105). Fewer patients with previous intensive treatment developed new cases of microalbuminuria with the reduction in odds by 59% (95% CI 39-73) although the difference in HbA1c was small between the groups after the end of the DCCT. The corresponding odds reduction for new cases of macroalbuminuria was 84% (95% CI 67-92), also in favour of previous intensive treatment.

Relations of glycaemic control to impaired GFR, creatinine and histologic lesions have also been reported. In the UKPDS there was a beneficial effect of intensive treatment on both the development of proteinuria and doubling of serum creatinine (90). A greater decline in GFR has also been associated with higher HbA1c levels (106). Moreover, intensive treatment in type 1 diabetes patients during 5 years after renal transplantation has shown a preventive effect on the development of glomerular lesions compared with patients randomised to standard glycaemic control (107). The importance of good glycaemic control has also been demonstrated in pancreas-transplanted patients where the patients generally reach normoglycaemia with absence of severe hypoglycaemias (108). There were apparent reversals of lesions of glomerulopathy ten years after pancreas transplantations returning to the normal range in several cases.

4.3 Macrovascular complications

In the UKPDS of type 2 diabetes there was no significant effect of intensive treatment on the risk of cardiovascular disease (CVD), although the effect on myocardial infarction was of borderline significance (90). Two recently presented clinical trials of type 2 diabetes with the aim of intensive therapy to reach as near normal blood glucose levels as possible could not either show any salutary effect on CVD with intensive treatment (109, 110). In fact, one of the trials was stopped after 3 years when the interim-analysis showed that the mortality was increased with intensive treatment (109). The effect of intensive treatment on CVD has not been possible to study in RCTs of type 1 diabetes. In the DCCT patients were only 13-39 years old and only 6 patients had a non-fatal myocardial infarction, stroke or cardiovascular death (111).

There are several epidemiologic studies of HbA1c and CVD. In the observational analysis of the UKPDS there was a significant relation between HbA1c and CVD (67). 1 percentage unit lower updated mean HbA1c reduced the risk by 14% (95% CI 8-21) for myocardial infarction, and 12% (95% CI 1-21) for stroke. At the 10-year follow-up of the UKPDS there were significantly fewer myocardial infarctions in the former intensive group although the difference in HbA1c was small after the end of the randomisation (112). The 11-year follow-up of the DCCT also showed a significant preventive effect of former intensive treatment on CVD (113). Former intensive treatment reduced the risks for any cardiovascular event by 42% (95% CI 9-63%) and by 57% (95% CI 12-79%) for non-fatal myocardial infarction, stroke or cardiovascular death. Most of the beneficial effect of intensive treatment on the risk of CVD was related to the decrease in HbA1c during the DCCT.

In a meta-analysis the relative risk increase was 1.18 (95% CI 1.1-1.26) for coronary heart disease and stroke with 1 percentage unit higher HbA1c in type 2 diabetes (114). In type 1 diabetes the relative risk for one percentage point increase in HbA1c was non-significant of 1.15 (95% CI 0.92-1.43). Only 3 studies of type 1 diabetes were included. In a prospective cohort of 23,751 type 1 diabetes patients the relative risk for mortality from coronary heart disease was several times higher than for a matched non-diabetic population (115). The follow-up period was up to 29 years and other risk factors, including those in the metabolic syndrome, were significantly lower than for patients with type 2 diabetes.

In 4,662 men from the general population the importance of HbA1c as a risk factor for mortality was prospectively examined (116). An increase in HbA1c by 1 percentage unit led to 28% greater risk for mortality independent of serum-cholesterol, age, blood pressure, BMI and smoking. This effect still existed after patients with diabetes, earlier myocardial infarction and stroke had been excluded. In the Diabetes Epidemiology Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) study it was shown that there is a relation of hyperglycaemia and risk of CVD in the normal range of blood glucose (117-119). There was a linear relation and no threshold between the level of blood glucose and CVD. Both fasting and postchallenge hyperglycemia were associated with risk of CVD (117).

A higher HbA1c level has been related to peripheral arterial disease (PAD) in several studies (67, 87, 120-122). In a meta-analysis of patients with type 2 diabetes there was a pooled relative risk for PAD of 1.28 (1.18-1.39) by 1 percentage unit higher HbA1c (114). In the observational analysis of the UKPDS the association between HbA1c and PAD was stronger than for myocardial infarction and stroke (67). One percentage unit lower updated mean HbA1c was associated to 43% (95% CI 31-53) lower risk for PAD.

The associations between HbA1c levels and risk of macrovascular complications in diabetes are complex. One possibility is that it takes a long time for the preventive effect of good glycaemic control on CVD to be established since a preventive effect was observed first during the follow-up of the UKPDS.

Chapter 5 Research methodology in studies of insulins and diabetic complications

5.1 Methodology when evaluating effects of insulins

In general in medicine the results of randomised clinical trials (RCTs) are regarded as the highest level of evidence when studying effects of different treatments (123). However, large observational studies from clinical practice most often yield the same results if rigorous eligible criteria, strict definitions of outcomes and large study settings are used. It is puzzling that trials of insulin glargine with similar types of patients and insulin regimens have shown as different results as no effect on HbA1c to significant positive effects (32-35, Table 2). In RCTs of type 1 diabetes the studies of the smallest size were those showing effect on HbA1c and hence lack of power of the analyses cannot explain the findings (33-34). Furthermore, the FPG goals were higher in these RCTs and the goals of titrations of insulin glargine could thus not either have caused the differences. One possible explanation is that the meal time insulins were carefully titrated according to optimisation schemes and there was a close contact with a team in the studies with positive effects (33, 34). The size of studies or goals of FPG cannot either explain the divergent results of RCTs in type 2 diabetes of insulin glargine (37-45).

Another factor that could possibly affect the divergent results is the non-blinded study design used in all trials of insulin glargine. This is probably an important aspect in trials of insulin treatments since the patients themselves are highly interactive with the dosage and titrations of insulins (6, 7). This is e.g. not the case when a pill of one dose is taken each day for reduction of blood pressure or blood lipids. Evaluations in clinical practice are therefore probably of greater importance for insulins than many other treatments.

In studies from clinical practice of insulin glargine and rapid-acting insulin analogues in principle all studies have been performed in the same centre with those designing the studies (Table 5). This has earlier been reported as a risk factor for getting misleading results in observational studies (123). There are risks for both treatment and assessment biases (124). In the majority of studies no control group has been used (Table 5). The necessity of control groups in observational studies is generally taken for granted in recommendations of observational studies (123). There is e.g. a risk of regression to the mean in studies of patients with poor glycaemic control (125). One example of an external factor that has earlier influenced evaluations of glycaemic control in clinical practice is disturbances in the laboratory method (126).

The need of research for predictors of antidiabetic agents has previously been addressed (127). However, in clinical trials as well as in clinical practice predictors of insulin effects have generally not been studied (32-35, 37-54). Predictors of insulin effects could be of use for recommendations in clinical practice and for suitable inclusion criteria in the design of future clinical trials. To examine predictors large patient materials are needed which could be an explanation in some cases why such studies have not been made.

Table 5: Studies of insulin glargine and insulin lispro in clinical practice

Study	Number	Multicentre	Controlled
Glargine			
Maia	49	No	No
Erickson	43	No	No
Schreiber	54	No	No
Haladova	136	No	No
Yamamoto-Honda	83	No	No
Lispro			
Lunt	284	No	Yes
Stocks	150	No	No
Chaterjee	221	No	No

5.2 Methodology when relating HbA1c to diabetic complications

In epidemiologic studies it is common to use the baseline value of a risk factor and relate it to later development of diabetic complications (114). It is also common to use a mean value of many recordings of the risk factor to get a more accurate estimate of the average level during the study period. In observational analyses of the UKPDS and the DCCT outcomes have been related to baseline HbA1c as well as updated mean HbA1c (60, 67). Updated mean HbA1c is a time-dependent covariate in a Cox or Poisson regression model. It could more simply be described as a mean value that is recalculated every time a new measurement of HbA1c is made. In the DCCT relations of retinopathy were also made to the standard deviation of HbA1c without adding any further predictability than the updated mean HbA1c (60). Some articles have questioned whether the use of baseline HbA1c could lead to lower predictability since only one value is used (128-130). In such a case this could influence risk estimates between HbA1c and diabetic complications. The updated mean HbA1c might not be a good predictor either since it gives the same importance to all historical HbA1c values wherever they in terms of time are situated. In review articles of HbA1c and diabetic complications the questions whether baseline or updated mean HbA1c are good predictors have not been further discussed (80, 131, 132). The influence of the frequency of HbA1c measurements, length of studies or definitions of outcomes on risk gradients between HbA1c and diabetic complications have not been dealt with either.

With commonly used statistical analysis of risk estimations in research today such as multiple, logistic and Cox regression time-dependent effects cannot be evaluated (133-135). The possibility of different influence for different risk factors on diabetic complications with time has not earlier been discussed in review articles of HbA1c and diabetic complications (80, 131, 132). In a similar way, it is possible that the salutary effects of some pharmacological treatments take time before they appear and that the beneficial effects can increase with time. Generally only the average effect of a treatment during the study period is presented (10, 67, 90, 133, 136, 137), but this may underestimate the benefit of an

intervention if the effect of a treatment actually increases with time. In clinical practice many patients receive chronic treatments, e.g. with antihypertensive and antidiabetic agents for much longer periods of time than the duration of the clinical trials.

5.3 Electronical tracking of data in clinical research

In studies from clinical practice of insulin lispro and insulin glargine data have generally been collected manually which could be the reason why relatively speaking small patient samples in the majority of cases have been used (Table 5). It might also explain why unicentre design has been made in all studies. Today there are patient record and quality improvement systems that can be tracked electronically (138-141). The corresponding information possible to obtain manually should preferentially be possible to search electronically such as medications, detailed information of risk factors and possible confounders. The electronical tracking could possibly make it easier to examine effects of insulins in more patients and from several care units leading to a more representative sample of the studied population and lower risk for random error.

Electronical tracking of data has e.g. earlier been used when evaluating treatments of cardiovascular disease (138, 140). In the field of anticoagulant therapy such studies have probably had a crucial role in reducing mortality and adverse events (140). By such studies it was demonstrated that greater cautiousness and lower doses should be given e.g. in patients with prostheses of the cardiac valves. Later reports have confirmed the results (142). Important for the findings were very large patient materials of several million blood samples and 3000 cases of mortality.

The enormous possibilities arising with electronical record systems in future research have earlier been recognized (138). However, it is important to recognise that the architecture of these systems is crucial to make electronical tracking of data possible. Different coordinated terminologies and functions handling coded content must therefore exist. Details to an extent of the level for eligibility criteria in RCTs should preferentially be possible to track.

Chapter 6 Aims

In this thesis we have used a patient record and quality improvement system with the possibility of electronic tracking of treatments and risk factors from clinical practice as well as publicly available data from the DCCT. We evaluated modern insulin analogues in clinical practice and developed research methodology to increase the understanding of the importance of HbA1c for diabetic complications. A term introduced in this thesis is the HbA1c variable which e.g. is a baseline HbA1c, or updated mean HbA1c, i.e. describes different weightings and combinations of HbA1c values. The specific aims were:

- 1) Estimate what effect on HbA1c is obtained when changing from NPH insulin to insulin glargine compared with continuing with NPH insulin in clinical practice.
- 2) Estimate what effect on HbA1c is obtained when changing from regular insulin to a rapid-acting insulin analogue compared with continuing with regular insulin in clinical practice.
- 3) Review what HbA1c variables, and statistical methods have been used in earlier studies of HbA1c and diabetic complications, and whether time-dependent effects have been evaluated. Moreover, from these data we also aimed at making compatible calculations of the predictive power of these HbA1c variables.
- 4) Create a model for an optimally predictive HbA1c variable, i.e. a variable that considers the total importance of HbA1c measurements at different levels and points of time. Evaluate by simulations whether the predictive power could possibly be much greater with an “optimal” HbA1c variable than the updated mean HbA1c.
- 5) If simulations show that more predictive HbA1c variables can exist, estimate the optimally predictive HbA1c variable and the temporal relationship between HbA1c and diabetic complications.

Chapter 7 Methods

7.1 Data sources

Diab-Base

Diab-Base is a patient record and quality improvement system from which extensive patient data of diabetes patients can be tracked electronically (8, 141). Similar systems exist for anticoagulant treatment, cardiology and chemotherapy (8, 140). From Diab-Base it is possible to track different treatments, doses of medications and the date when treatments have been changed for individual patients. The system also provides extensive information of the HbA1c levels measured in clinical practice, grade of microalbuminuria, blood pressure, blood lipids and other common risk variables and laboratory markers of diabetes. Information of retinopathy, neuropathy, nephropathy, foot ulcers, amputations, myocardial infarction, stroke and autonomic complications can also be tracked in the system. A special function has been developed for the system to download information. Via this module selections can easily be made by choosing which parameters should be downloaded to Microsoft Excel. Raw data is then automatically downloaded in two separate files. One file describes stationary parameters and the other file includes dates and parameters that are followed over time, i.e. variables at each visit. Examples of stationary parameters are year when diagnosis was made and point of time when insulin was initiated since these do not change over time. Of longitudinal parameters are e.g. laboratory values, insulin doses, grade of retinopathy and different medications included. After raw data has been downloaded special programs must be made to extract the data of interest for the specific research questions. For simpler and more general questions such as average levels of HbA1c, blood pressure, blood lipids, patients with poor glycaemic control, proportion of patients treated adequately due to guidelines predefined modules exist. An example of adequate treatment to guidelines is how many of patients with microalbuminuria are treated with ACE-inhibitors or angiotensin-II-blockers.

Diabetes Control and Complications Trial (DCCT)

We have also used data from the DCCT which are publicly available (9). The aim of the 9-year DCCT study of 1,441 patients with type 1 diabetes was to compare the effects of intensive and conventional blood glucose treatment on the development and progression of the complications associated with diabetes (10). At the time of inclusion in the DCCT the patients were 13 to 39 years old and their duration of type 1 diabetes was 1 to 15 years. When randomization took place, the participants had no advanced micro or macrovascular complications resulting from diabetes. They were divided into two strata. The primary prevention cohort (n = 726) showed no evidence of retinopathy according to fundus photography and urinary albumin excretion rate (UAER) <40 mg/24 h. The secondary intervention cohort (n = 715) had minimal to moderate retinopathy and UAER <200 mg/24 h.

Patients were randomized to intensive therapy with multiple daily insulin injections or conventional insulin therapy. Intensive therapy was constituted by multiple daily insulin

injections or CSII together with frequent measurements of blood glucose. Conventional therapy was 1 or 2 insulin injections per day.

HbA1c levels were measured every month in the intensively treated group and quarterly in the conventionally treated group. Fundus photographs were taken every six months and measurements of albuminuria made every 12 months. Development of retinopathy was defined as 3 steps worsening of retinopathy on the ETDRS scale that was sustained a half year later.

7.2 Evaluation of insulin glargine in clinical practice

Information was collected from Diab-Base. All patients with NPH insulin changing to insulin glargine as basal insulin during multiple daily insulin injections (MDI) were included. Controls continuing with NPH insulin during a similar time period were also examined. In total data from 15 outpatient diabetes clinics were collected. For a patient to be included an HbA1c value before the change to insulin glargine must exist as well as an HbA1c value at least 60 days after the change.

Evaluations of insulin glargine in subgroups of patients

Possible positive predictors of a reduction in HbA1c associated with a change to insulin glargine were examined. The following potential predictors were examined: age, gender, duration of diabetes, type of diabetes, smoking, metformin use, insulin requirement (units insulin/kg body weight), number of basal doses per day, BMI and HbA1c at baseline. The same predictors were studied for the controls continuing with NPH insulin. The effect of changing to insulin glargine was yielded from comparisons of the effects on HbA1c for the corresponding subgroup of patients among the controls.

Evaluations of average effects of insulin glargine

The average effect of insulin glargine was estimated by comparing the HbA1c values before change to insulin glargine and after the change. To control for regression to the mean the HbA1c values for patients changing to insulin glargine were controlled 2 years before the change. To control for external influences change in HbA1c for controls continuing with NPH insulin was also studied.

7.3 Evaluation of insulin lispro in clinical practice

Information was collected from Diab-Base. The effect of insulin lispro on HbA1c over a 5-year period in 14 outpatient clinics was studied. The time period was chosen when most patients had not changed basal insulins but only meal time insulins. Disturbances in laboratory methods can effect evaluations of HbA1c in clinical practice (126). One possible way to reduce such risks could be to evaluate HbA1c values at the same point of time for different treatments. Another way could be to follow patients longitudinally during a long

period before and after the change of treatment and examine that HbA1c is stable before and after the change. Another important factor could be that patients have been on insulin treatment for a longer period before baseline and are thus used to titrations and the insulin sensitivity is stabilised.

In this study patients with NPH insulin and regular insulin who either continued with this regimen or changed to insulin lispro during a 5-year period were studied. Patients should have been on the treatment for more than 300 days to be included. At least one HbA1c value should exist for all patients at baseline in 1996 and one in 2001. The last HbA1c value should be at least 2 months after the change to insulin lispro.

Evaluations of average effects of insulin lispro on HbA1c

The average effect was studied in two ways. The change in HbA1c when changing to insulin lispro was adjusted for controls with the same HbA1c level. Adjustments were also made for differences in BMI, age, gender, diabetes duration, duration of insulin treatment, the year for change of insulins, type of diabetes, smoking, weight, daily insulin need and number of insulin doses. The average effect was also studied by examining the HbA1c levels longitudinally before the change of treatments and the effect of the change in treatment.

Evaluations of insulin lispro in subgroups of patients

The following possible predictors of a greater effect of insulin lispro on HbA1c were evaluated: age, gender, BMI, diabetes duration, duration of insulin treatment, the year for change of insulins, type of diabetes, smoking, weight, daily insulin need and number of insulin doses.

7.4 Earlier used HbA1c variables and their predictive abilities

Literature search

Medline (www.pubmed.gov) searches were performed with the following search terms. *HbA1c* and *glycosylated haemoglobin* were each combined with *retinopathy*, *microalbuminuria*, *nephropathy*, *neuropathy*, *myocardial infarction*, *stroke*, *ulcer* and *amputation*.

Included articles

Priority was given to clinical trials, followed by other prospective studies and, in special cases, retrospective studies. All publications of clinical trials in English including more than 75 patients and other prospective studies on 100 patients or more were included if they focused on the relation between HbA1c and diabetic complications. Publications of retrospective studies were included if they included any risk variable apart from the baseline, mean or updated mean HbA1c values. All publications in which it was not possible to judge

from the abstract whether the studies were prospective or retrospective were also studied, but were not included in the study if they did not provide any useful information on the HbA1c variables. In total 8,000 abstracts, initially 130 full text articles and finally 97 full-text articles were reviewed.

Recorded parameters

HbA1c variables used, length and size of studies, age and gender and diabetes duration of participants, frequency of HbA1c measurements, statistical methods, significant risk factors for complications and frequency of HbA1c measurements were recorded. Study type and type of risk estimate used were also registered. All information concerning temporal relationships between HbA1c and complications was also recorded.

Comparisons of the predictive power of earlier used HbA1c variables

To compare the predictive power of earlier used HbA1c variables the quantity gradient of risk per standard deviation was used which earlier has been recommended when comparing risk variables (143, 144). A criterion for comparing variables was that their risk estimates were presented in one and the same study i.e. under the same circumstances. We also registered the risk estimations used in the individual studies.

7.5 Simulations of HbA1c variables

Model of analysis

The model of analysis was based on the fact that each diabetes patient has a continuous HbA1c curve. An infinite set of HbA1c variables can be constructed from a continuous HbA1c curve. We assumed that in this infinite set of HbA1c variables there is one “optimal variable”, which takes into account the way in which different levels of HbA1c at different times influence the risk of developing diabetic complications. Contrary to this, the updated mean HbA1c implies that the HbA1c value has the same importance at all points in time. Thus, we constructed HbA1c variables that we believed could be realistic candidates for the optimal HbA1c variable. These variables included scenarios that we believed realistic of how HbA1c during a short time interval affects diabetic complications now and in the future. Hence, no real diabetic complications were used in the model. A mathematical relationship between the predictive power of two variables and the correlation coefficient between them was the basic tool for the comparisons.

The study consisted of three main parts:

- 1) Simulation of HbA1c values that can be linked together to form a continuous HbA1c curve.

- 2) Construction of candidates for the optimal HbA1c variable by describing how each short time interval of the continuous HbA1c curves affects diabetic complications now and in the future.
- 3) Comparison of the predictive power of the HbA1c variables constructed and the updated mean HbA1c.

Simulation of continuous HbA1c curves

Continuous HbA1c curves were simulated for 10,000 hypothetical diabetes patients. Monthly HbA1c values were simulated on the basis of HbA1c values from clinical practice and connected by lines to form the continuous curves (Paper IV, Fig. 1). The values from clinical practice were 65,534 HbA1c measurements from 12,980 diabetes patients collected from Diab-Base and were used to determine the correlation coefficient for two values from the same individual as a function of the time interval between them. Using this function made the simulations more realistic.

Construction of HbA1c variables

We assumed that the maximum harmful effect of HbA1c on diabetic complications is not necessarily manifested at the same time as the current value of HbA1c. We constructed HbA1c variables consisting of an integral of the product of two functions g and f depending on the continuous HbA1c curves. The function g reflected how the effect of HbA1c persisted by time and f the relation between the level of HbA1c and diabetic complications.

The function g comprised three parameters: 1) time to maximum effect on the development of diabetic complications, 2) the rate of increase in the effect until the maximum is reached, and 3) the rate of decrease in the effect after the maximum (Fig. 3, Paper IV).

The persistent effect profiles applied to the simulated continuous HbA1c curves are given in Table 1 Paper IV. Completely flat effect profiles were investigated, i.e. a short interval of HbA1c has a consistent effect on diabetic complications. Profiles with a maximum effect on diabetic complications after 0.5, 2 and 4 years were also tested. The increase in the effect to these maxima was widely varied as was the following decreasing phase.

The function f comprised a fourth parameter, which reflected that the risk increase could be different in the interval above 8.7% and 6.0-8.7% (DCCT-standard). The value 1 of this parameter corresponds to equal risk increase in these two intervals.

Comparison of the predictive power of the updated mean HbA1c value and the HbA1c variables constructed

As a measure of the goodness of the predictors we used the gradient of risk per 1 standard deviation. Due to the existence of a mathematical relationship, which we have derived, between the gradients of two variables and the correlation coefficient between them, we could perform the comparison between the gradients by studying the correlation coefficients between them.

7.6 The temporal relationship between HbA1c and retinopathy in the DCCT

Relating glycaemic exposure to retinopathy

We used the HbA1c values which were measured every month in the intensively treated group and quarterly in the conventionally treated group as well as scores of fundus photographs which were taken every six months. We defined progression of retinopathy as a three-step worsening on the final version of the Early Treatment Diabetic Retinopathy Study (ETDRS) scale which comprises 23 steps of retinopathy grading (64, 145). When using the baseline and updated mean HbA1c levels the change in time to progression of retinopathy is not taken into account. To allow the temporal variation to be studied we have developed a simple function dependent on time, $g(t)$ which reflects how the HbA1c level contributes to later risk of progression of retinopathy (Paper IV, Fig.3). It includes 3 unknown parameters: the first reflecting the time from a short glycaemic exposure to reach maximum effect on retinopathy, the second reflecting how the effect increases and the third how it decreases.

By using all HbA1c values a polygon can be created for each individual, which can be used as an approximation of the continuous HbA1c curve for that individual. The function $g(t)$ is part of an integral in which these HbA1c values are also included, which we here refer to as the optimal HbA1c variable. In the presented models when relating different HbA1c variables to retinopathy, age, gender, diabetes duration, treatment group and the influence of time have been studied.

7.7 Statistics

Evaluations of insulin glargine

Multiple regression was used to assess predictors for both the insulin glargine group and controls. Significant predictors of insulin glargine effect were used to create a multiple regression model to predict the change in HbA1c. The same procedure was used for controls. The difference in the regression functions gave the effect of changing to insulin glargine compared to continuing with NPH insulin. The difference between baseline HbA1c and the last HbA1c value was not normally distributed in any of the groups and hence transformations

were made in both groups. The regression coefficients for gender in the two groups were compared with a test based on the normal distribution.

Evaluations of insulin lispro

Multiple regression analysis with HbA1c as dependent variable was used for the whole cohort. Among the independent variables the 0/1-variable indicating a change of treatment to insulin lispro together with the interaction of this variable was included.

Calculation of gradient of risk per 1 SD for HbA1c variables

When comparing the predictive power of HbA1c variables used in earlier studies the quantity gradient of risk per standard deviation was used. Gradient of risk per standard deviation is defined as $\exp(b \cdot SD)$ where b is the beta coefficient derived from a Cox or Poisson regression analysis and SD is the standard deviation of the HbA1c variable in the population of diabetes patients studied. For example, a gradient of 1.30 implies that the risk of complications increases by 30% when the HbA1c variable increases by 1 standard deviation. The distribution of the HbA1c variable must be normal and transformations were made if this was not the case.

Simulation of continuous HbA1c curves and comparison of constructed HbA1c variables and updated mean HbA1c

A general measure of the goodness of a predictor is the *gradient of risk per standard deviation*, which is the relative increase in the hazard function when the value of the variable is changed by 1 standard deviation in the direction of risk. This allows comparisons between the goodness of different predictors. The best predictor of the risk of developing a complication based on the complete HbA1c curve during the follow-up period is assumed to be a variable calculated by superimposing an infinite set of curves. Considering one such curve, the corresponding function is assumed to be the product of a function f of a single value of HbA1c at time t and a function g of the time since t .

We assume that f is continuous everywhere and piece-wise linear. For HbA1c values below 6.0 the function f is assumed to be 0, and between 6.0 and 8.7 to increase at a rate b . Above 8.7, the rate of increase is assumed to be b multiplied by a factor c . In the tables below, the factor c is referred to as “Parameter in function f ”.

The correlation coefficient between a certain HbA1c value and later values was calculated using linear regression. Wiener processes, which have Markovian properties, were used to simulate HbA1c values at monthly intervals (146). It was assumed that HbA1c values without measurement errors could be well approximated by a Wiener process. For each hypothetical patient we calculated the value of the HbA1c variable as an integral comprising the functions g and f (Paper IV, Supplementary material) at the end of the follow-up period; the updated mean was also calculated. The correlation coefficient between the two variables was then calculated. Finally, we applied the relationship given below to compare the gradients of risk.

If a predictor **A** comprises all predictive information that another predictor **B** comprises, then the following relationship between their gradients is true, provided that **A** and **B** have normal distributions:

$$\text{Gradient of A} = (\text{Gradient of B})^{1/|\rho|}$$

where ρ is the correlation coefficient between A and B.

Estimations of the optimal predictor of retinopathy in the DCCT

We studied a group of functionals based on a theoretical consideration of how the HbA1c level could influence the risk. We assumed that during a short time interval, Δv , around the time v , a patient has a usual or transformed value of HbA1c denoted $y(v)$. We define time $t=0$ as the time of diagnosis of diabetes. At a point in time t , where $t > v$, the contribution to the risk is given by: $y(v) \cdot \Delta v \cdot g(t-v)$. The function g , which is unknown, first increases and then decreases. The total contribution to the risk at time t is given by:

$$\int_0^t y(v) \cdot g(t-v) dv ,$$

which is referred to below as the optimal variable. The function g is determined by 3 parameters:

$$g(t) = \exp(B1 \cdot \min(t, B2) + B3 \cdot \max(t-B2, 0))$$

If $B1$ is positive and $B3$ negative, the maximum is obtained for $t=B2$. Thus $B2$ is the time at which the effect of HbA1c is a maximum. After the maximum, the contribution to the risk declines at a rate determined by $B3$. We define the function $y(v)$ to be: $\max(\text{HbA1c}(v) - B4, 0)$, where $\text{HbA1c}(v)$ is the value of HbA1c at time v . This means that the function is determined by only one constant, $B4$. There are thus 4 unknown constants in our functional. The risk model has 6 unknown constants, giving a total of 10. We have 532 retinopathy events with which to estimate these 10 parameters (i.e. more than 50 per parameter).

The hazard functions of the progression of retinopathy were estimated using Poisson regression (147). The baseline value, the updated mean and the optimal variable were included in the analyses, together with age, sex, diabetes duration, treatment group and time since entry into the trial. Interactions between the HbA1c variables and time since entry were studied. This method can be applied to events whose time of occurrence is exactly known, such as myocardial infarction and death, as well as for retinopathy where the point in time is described by an interval.

Chapter 8 Results

8.1 Effects of insulin glargine in clinical practice

In total 4,001 patients with type 1 or type 2 diabetes were included from 15 outpatient diabetes clinics. There were 1,639 patients with insulin glargine followed during 10 months (IQR 6-15) and 2,362 controls with NPH insulin followed over 12 months (IQR 9-15). All patients in both groups were also treated with meal time insulin due to the inclusion criteria.

Effects of insulin glargine in subgroups of patients

Positive predictors for a reducing effect on HbA1c of insulin glargine were male gender ($p<0.001$), low BMI ($p<0.01$) and high HbA1c at baseline ($p<0.001$). In the controls only high HbA1c at baseline was a positive predictor for a reduction in HbA1c ($p<0.001$). Male patients with BMI 25 had a beneficial effect after adjustments with the controls of 0.26 percentage units calculated from an HbA1c level of 8.0% with Mono-S-standard (Paper I, Fig. 1). Women with BMI 30 had no effect of a change to insulin glargine. The multiple correlation coefficient for patients changing to insulin glargine was 0.51 (SD around the regression function 0.77) and in controls -0.38 (SD 0.858). When comparisons were made with the controls gender had a significant greater influence on HbA1c for patients with insulin glargine ($p=0.0053$).

Average effect of insulin glargine on HbA1c in clinical practice

For patients changing to insulin glargine HbA1c decreased on average by 0.18 percentage units from 7.49%(SD 1.6) to 7.31% (SD 1.3) ($p<0.001$). There was no difference in the HbA1c level two years before baseline on 7.48% (SD 1.6) compared to 7.49% (SD 1.6) at baseline. Insulin requirement increased from 0.64 ± 0.25 to 0.65 ± 0.25 U/kg/day ($p<0.01$). The number of basal insulin injections decreased from 1.4 ± 0.5 to 1.1 ± 0.3 ($p<0.001$). Body weight increased from 75.9(SD 14.7) to 76.4 (SD 14.9) kg ($p<0.001$).

8.2 Effects of insulin lispro in clinical practice

We studied in total 1,069 diabetes patients with NPH insulin as basal insulin and at least 3 daily injections with regular insulin of whom 423 changed insulin to lispro during a 5-year period. There were 646 controls continuing with regular insulin.

Average effect of insulin lispro on HbA1c

Patients changing to insulin lispro decreased on average 0.19 percentage points in HbA1c compared to controls continuing with regular insulin. A beneficial effect of insulin lispro was

also indicated by the fact that patients had the same HbA1c level during a long period with regular insulin but then decreased when changing to insulin lispro.

Effect of insulin lispro in subgroups of patients

Patients with high HbA1c had the most pronounced effect of insulin lispro even after controlling for regression to the mean (Paper II, Fig. 1). BMI was a predictor for greater changes in HbA1c in the whole cohort.

8.3 Predictive ability of HbA1c variables

HbA1c variables and statistical methods in earlier studies

The most commonly used HbA1c variables were: the baseline value, the mean and the updated mean HbA1c (Table I, Paper III). Other variables used were the logarithm of the updated mean, the standard deviation, the slope (annual average change), the initial decline (change during the first year), the final value, and the change in HbA1c between baseline and the fourth year.

The most commonly used statistical methods were Cox regression, logistic regression and multiple regression analyses.

Earlier used HbA1c variables with high predictive ability

The updated mean, logarithm of the updated mean and mean HbA1c were found to have greater predictive power than baseline HbA1c (Paper III, Table 2 and 3). The slope, final value, SD, initial decline and change of HbA1c did not add any further information.

Influence on predictive ability of study length and frequency of HbA1c measurements

The predictive power of the mean or updated mean HbA1c became stronger with longer study lengths. There was a persistent effect over several years between HbA1c values and diabetic complications. Measurements of HbA1c varied from a single value to measurements each month. However there were no estimations found of how the frequency of HbA1c measurements influenced the predictive ability.

8.4 Correlation coefficients between simulated HbA1c variables and updated mean HbA1c

The correlation coefficient between the predictive power of the updated mean HbA1c and the constructed variables, which take the time-dependent effect of HbA1c into account, ranged

from 0.53 to 0.78 (Paper IV, Table 1). For a certain diabetic complication with a gradient of risk per 1 SD higher updated mean HbA1c of 1.3, a correlation coefficient of 0.5 means that an optimal variable would instead have the gradient 1.69 (Paper IV, Fig. 4). For another complication, when the gradient is e.g. 2 per SD higher updated mean HbA1c, a correlation coefficient of 0.5 instead means that an optimal variable would have the gradient 4. A gradient of risk per 1 SD higher of an HbA1c variable of e.g. 1.3 for a certain diabetic complication means that the risk increases by 30% when the HbA1c variable increases 1 SD and a gradient of 4 that the risk increases by 300%.

8.5 The temporal relationship between HbA1c and retinopathy

Time course of HbA1c and progression of retinopathy

We estimated the temporal relationship between HbA1c and the risk of development of retinopathy (Paper V, Fig. 1). The function $g(t)$, reflecting how the level of HbA1c during a short time interval relates to the development of retinopathy over a longer time, shows that the blood glucose level, measured as HbA1c, has a direct effect on the risk of development of retinopathy. The effect increases for 2.4 years to the maximum harmful effect, which is 2.8 times greater than the initial effect.

Change in momentary risk over time

The temporal relationship between HbA1c and the development of retinopathy can be used to calculate the change in momentary risk over time. The hazard ratio for HbA1c 8% versus 7% was e.g. 1.05 at 1 year, 1.63 at 5 years and 2.25 at 10 years (Paper V, Fig 2).

Interaction with time

Both the traditional variable (the updated mean HbA1c) and the optimal HbA1c variable (including the temporal relationship of HbA1c and retinopathy) showed a significant interaction with time ($p < 0.001$). A significant interaction was also found between treatment group and time when HbA1c was not included in the model ($p < 0.001$). This interaction shows that the hazard ratio for intensive versus conventional treatment decreases with longer follow-up time.

Predictive ability

The predictive abilities of baseline HbA1c, updated mean HbA1c, the optimal HbA1c variable and updated mean HbA1c including the interaction with time were calculated (Paper V, Table 1). For HbA1c 8% versus 7% the optimal HbA1c variable predicted 92% greater risk for progression of retinopathy over 6 years (mean follow-up time in the DCCT = 6.5 years). The updated mean HbA1c value including the interaction with time predicted 86% greater risk, the traditional updated mean HbA1c a 50% increase in risk, and the baseline HbA1c a 30% increase. The predictive ability of the new variables increased with increasing follow-up

time. The log likelihood value for the optimal variable was higher than for the updated mean HbA1c value including the interaction with time, illustrating a better fit of the optimal variable.

Numbers needed to treat

We calculated NNT for different 3-year intervals when the HbA1c level is reduced by 1 per cent unit from different HbA1c levels, by 2 per cent units from 9% to 7%, which was the case in the DCCT, and for a relatively small decrease in HbA1c from 8.3% to 8% (Paper V, Table 2). NNT decreases rapidly with time and is e.g. 273.7 for the first three-year period and 2.1 for the last three-year period when HbA1c is reduced from 9% to 7% during 12 years.

We calculated NNT for 7% versus 8% when both patient groups had been on 8% during the previous 5.8 years (median diabetes duration in DCCT) and when the patients with HbA1c 7% had been on the same level already from diagnosis. When the previous HbA1c had been on 8% instead of 7% the NNT from 5.8-8.8 years for HbA1c 8% versus 7% was 198% higher (33.4 versus 11.2) and 74% higher for the period 8.8-11.8 years (9.4 versus 5.4).

Design of future trials

The temporal relationship between HbA1c and retinopathy also makes it possible to design study scenarios. We estimated a 3-year scenario of a randomized study when improving the glycaemic control from HbA1c 8% to 7% (Paper V, Fig 3). There would be 16% more events of retinopathy after 3 years. If the randomized groups would have had the difference in glycaemic control already several years before the study, there would instead be 86% more events during the same 3-year period and circumstances. Hence, the study must be designed for a longer period, so the impact of pre-study glycaemic control is reduced. A new scenario over a longer period of time could be made with the help of the temporal relationship of HbA1c and retinopathy.

Chapter 9 Discussion

9.1 Insulin glargine and lispro improve HbA1c in clinical practice

We estimated the effects on HbA1c of modern insulin analogues in clinical practice (Paper I and II). Insulin glargine reduced HbA1c significantly by 0.18 percentage units and insulin lispro by 0.19 percentage units in comparison to NPH insulin and regular insulin respectively. Earlier studies from clinical practice as well as RCTs have shown divergent results concerning effects on HbA1c for both insulin glargine and insulin lispro (32-35, 46-54, Table 2). In the present studies many more patients were evaluated than in earlier studies from clinical practice, multicenter design and control groups were used which have often been lacking (Table 5). The present study of insulin glargine included 4,001 patients from 15 clinics and examinations of insulin lispro were made in 1,069 patients from 14 care units. We therefore believe the present studies give a good estimation of what changes in HbA1c could be expected when using these insulins in clinical practice. We included patients with multiple daily insulin injections (MDI) and in principle all patients were treated in specialist care units. Hence, the study of insulin glargine is not representative for patients with insulin glargine in monotherapy without MDI-treatment.

9.2 Effects of insulin analogues in subgroups of patients

In earlier studies from clinical practice mainly type 1 diabetes patients have been studied. In the present studies there was no significant difference in the effect of insulin glargine or lispro for type 1 or type 2 diabetes patients. However, for insulin glargine there was a larger effect for men and for insulin lispro for patients with high HbA1c. A possible explanation is that patients with high HbA1c have better compliance with rapid-acting insulin analogues since they are taken in close connection to their meals. BMI led to a greater change in HbA1c for the whole cohort in the study of insulin lispro, which was also observed for insulin glargine. This might be due to lower insulin resistance and could indicate the need of careful optimisation in these patients.

Predictors of treatment effects could be used in the design of clinical trials. A clinical trial in a subgroup of patients with a larger effect will be easier to carry out, more beneficial for the patients and motivate an introduction in clinical care. In our study of insulin glargine we show that if predictors are used in the design of a clinical trial less than 10% of patients in a general study population is needed. According to the results of our studies, 2x123 male patients with HbA1c above 8.0% and $BMI \leq 28$ are needed to reach the power 80% to detect an HbA1c difference of 0.33 percentage points. If no selection is made 2x1,410 patients are needed to reach the power 80% to detect a difference of 0.10 percentage points.

9.3 The temporal relationship between HbA1c and retinopathy

The momentary or current risk describe the harmful effect of impaired glycaemic control that exists right now, i.e. the risk for a worsening of retinopathy at a specific moment in time. It was earlier not known that the previous glycaemic control is more deleterious than the present control for the current risk of progression of retinopathy. We show that an exposure to HbA1c during a short time interval 2.4 years ago has the strongest influence on current risk for progression of retinopathy, 2.8 times greater than the present exposure (Paper V, Fig. 1). HbA1c values up to 4.9 years ago had greater influence than the current values. The temporal relationship was estimated from data of the DCCT. A function $g(t)$ was created which describes how HbA1c during a short interval affects the momentary risk at different later points of time. By an optimisation procedure using all HbA1c values and endpoints of retinopathy an optimal predictive power of an HbA1c variable where $g(t)$ was included was sought. The parameters of $g(t)$ for the optimally predictive HbA1c variable were assumed to reflect the temporal relationship between HbA1c and retinopathy. The temporal relationship has several implications which will be discussed below.

9.4 The momentary risk of retinopathy increases with time although HbA1c is constant

Our research model describes the momentary risk of retinopathy as a composite of the effects from all HbA1c values until the current point of time. Since the HbA1c values had a long persistent effect on retinopathy the influence from more and more HbA1c values will appear with time. For another risk variable which has not a persistent effect the momentary risk will not increase with time. The value of the hazard function, describing the momentary risk, was for an HbA1c-level of 8% 11 times greater 5 years after diagnosis than after 1 year (Paper V, Fig. 2). After 12 years the value of the hazard function was 78 times greater than after 1 year. The dramatic importance of previous glycaemic control on current risk implies that the importance of the HbA1c level is underestimated in earlier studies since the effect increases far beyond the end of the studies. The increase of the current risk with time also explains why diabetes duration usually appears as a potent predictor of diabetic complications.

9.5 Low HbA1c levels prevent retinopathy to a larger extent than earlier estimations have shown

When estimating the importance of HbA1c in preventing diabetic complications studies have most commonly used a baseline HbA1c value (Paper III, Table 1). We also showed that in earlier studies a mean or updated mean HbA1c have led to greater predictability than a baseline value (Paper III, Table 2 and 3). A recent analysis of the DCCT shows that the standard deviation of HbA1c added predictive power besides the updated mean contradictory to earlier analyses (148). In meta-analyses several included studies have used baseline HbA1c which hence will underestimate the pooled relative risk (114). No earlier used HbA1c variables have included time-dependent effects as presented here. One analysis of the DCCT made a risk estimation of how much 10% lower HbA1c (e.g. 7.2% vs. 8%) reduces the risk for progression of retinopathy (60). Hence, these estimations describe how much the average

reduction in retinopathy is obtained when lowering HbA1c during 6.5 years from an earlier high level of HbA1c. However, the prestudy glycaemic control will contaminate the beneficial effects by intensive treatment leading to underestimations.

We estimated the risk of progression of retinopathy if the difference in HbA1c is obtained already from diagnosis, i.e. 5.8 years before the study which was the median duration of diabetes in the DCCT. The risk increase of retinopathy associated with HbA1c 8% instead of 7% during the period from 5.8 years and 6 years onwards from diagnosis then led to 92% greater risk for progression of retinopathy instead of only 50% when updated mean HbA1c was used (Paper V, Table 1). If considering a longer period of time of 10 years than the DCCT of 6.5 years, the risk increase becomes greater of 130%. Hence, we have found that when estimating risk for retinopathy for a certain HbA1c level the risk estimate must be set in a perspective of how long time it is obtained, at which period after diagnosis and the level of previous glycaemic control. Hence, in meta-analyses not only studies using baseline HbA1c lead to underestimations of the pooled relative risk but also those using mean or updated mean HbA1c.

9.6 Numbers needed to treat to prevent retinopathy when lowering HbA1c

From the hazard functions for progression of retinopathy for HbA1c 8% versus 7% (Paper V Fig. 2) and similar functions for other HbA1c levels numbers needed to treat (NNT) to prevent retinopathy were estimated. The increasing effect of HbA1c with time became even more evident with these estimations (Paper V, Table 2). When e.g. HbA1c is lowered from 9% to 7% from diagnosis, the NNT is 274 for the first 3-year period but only 2 for the period 9-12 years. We showed that the dramatic decrease in NNT with time is due to two factors. Firstly the previous HbA1c levels have an effect also at a later point of time leading to a much more preventive effect during a later time period. Secondly the events of retinopathy become more frequent with time. The exact individual respective contribution of these two factors was not evaluated and is probably complex and differs for different time periods. However, we evaluated that the previous glycaemic exposure is of crucial importance and not only the increased frequency of events. The NNT was e.g. 66% lower for the 3-year period from 5.8-8.8 years after diagnosis if an HbA1c difference of 7% versus 8% had been obtained already from diagnosis and not only during the 3-year period.

9.7 Retinopathy appears in spite of good present glycaemic control

In clinical practice it is sometimes seen that patients develop retinopathy although good glycaemic control is obtained. There were 10% of patients developing retinopathy in the DCCT although very good glycaemic control existed (61). In that analysis baseline HbA1c as a measure of prestudy glycaemic control could explain some of these cases. However, since we here illustrate that the HbA1c values 2.4 years ago have the largest deleterious effect on current retinopathy and that values up to 5 years ago are of greater importance than present values, baseline HbA1c is a very rough measure of prestudy glycaemic control. Many of the retinopathy lesions appearing during good glycaemic control can therefore be due to impaired prestudy glycaemic control. In fact it is possible that most of these cases are explained by

poor glycaemic control that appeared before the study. In clinical practice and clinical trials glycaemic control can hence probably explain many cases of retinopathy that appear although present glycaemic control is good.

9.8 Which HbA1c reduction is clinically relevant in preventing diabetic complications?

We estimated the NNT for the period 9-12 years after diagnosis to 12.7 when HbA1c was reduced from 8.3% to 8% from diagnosis (Paper V, Table 2). It means that in Sweden with 9 million inhabitants and a diabetes prevalence of around 4% that roughly 28,300 fewer patients during this 3-year period would avoid progression of retinopathy if such an HbA1c reduction was obtained from diagnosis. It is then assumed that the effect is similar for patients with type 2 diabetes. With the knowledge that the risk gradient between HbA1c and nephropathy, neuropathy and amputations is equally steep as for retinopathy and that HbA1c possibly is of importance also for myocardial infarction and stroke (67), we believe an HbA1c difference of 0.2-0.3 percentage points should be considered clinically relevant.

In several power analyses in studies of insulin a clinically relevant difference of 0.3 percentage points of HbA1c is used (33, 34). As far as we know there has been no scientific basis for setting this limit, but probably made by intuition. Our findings support that this difference is clinically relevant and we believe that antidiabetic agents and other aids with a clear and reproducible effect of lowering HbA1c by 0.2 percentage points in both trials and practice should be given high priority. Hence, we also believe that power analyses should use this limit when searching for new treatment options.

9.9 Support of time dependent effects of HbA1c but not for blood pressure

In the UKPDS there was besides randomization to intensive glycaemic control a subgroup randomized to tight blood pressure control (90, 149). There was no beneficial effect seen on CVD by intensive glycaemic control but there was a substantial preventive effect by tight blood pressure control. Hence, the conclusions have for a long time been that blood pressure control might be more beneficial in preventing CVD than intensive glycaemic control. Shortly after the end of the randomization HbA1c and blood pressure levels became similar for the previously randomized groups but the patients with previously low levels of HbA1c developed fewer CVD events during the next ten years (112). However, the beneficial effect of tight blood pressure control deteriorated and there was no effect during this period (150). The effect of glycaemic exposure thus seems to increase with time whereas the salutary effect of blood pressure is more direct and rapidly disappears.

The EDIC studies also support a persistent effect of good glycaemic control on retinopathy, nephropathy and CVD (93, 105, 113). Moreover, in a study of patients with pancreas transplantations normalising their HbA1c levels there was no effect on glomerular lesions of the kidneys after 5 years but after 10 years typical lesions of diabetic nephropathy on biopsies had disappeared for several patients (108). The phenomenon we observed in the DCCT was also predicted in our previous simulation study from an independent patient material (Paper

IV). Finally in our analyses the presented model showed a much better fit than a traditional model illustrated by loglikelihood values (Paper V, Supplement).

9.10 Temporal relationships an important tool in design of clinical trials of HbA1c and diabetic complications

Two recently presented studies randomizing patients to intensive treatment as close to normal HbA1c as possible failed to demonstrate any preventive effect on CVD (109, 110). We understand after the findings in the follow-up of the UKPDS that this might be due to too short study settings (112). We here illustrate that scenarios can probably be better anticipated by estimations of the temporal relationship between HbA1c and diabetic complications. We show how events of retinopathy in two randomized groups considerably differ in a 3-year study depending on the prestudy glycaemic control (Paper V, Fig. 3). With the method we can estimate probable scenarios for different prestudy glycaemic control, differences in HbA1c during the trial and length of the study. If estimating the temporal relationship between HbA1c and CVD similar scenarios could be made for trials of intensive treatment and CVD. Such estimations could probably be of importance in the design of future trials and explain the absence of beneficial effects in previous studies. If e.g. HbA1c values many years back have the greatest effect on current risk of CVD, the trials must be designed for long periods of time.

9.11 Low HbA1c more important in preventing neuropathy, nephropathy and macrovascular complications

In simulations we show that if HbA1c during a short time interval has a persistent effect on progression of a diabetic complication the updated mean HbA1c used in earlier studies leads to large underestimations of the importance of good glycaemic control. Since the evidence is strong for a persistent effect of HbA1c on nephropathy, neuropathy and macrovascular complications, we are confident that a large underestimation of the glycaemic control also exists for these complications (Paper III, 112, 151). The predictive power of HbA1c could be up to 100% higher which was the case in retinopathy. In the simulations the persistent effect was widely varied and had its peak at different points of time. The time until the effect became less than the initial effect varied from 0.75 to 20 years. In all scenarios, only the existence of a persistent effect implied that an updated mean HbA1c led to considerable underestimations of the importance of the glycaemic control (Paper IV Table 1). Besides the predictive ability the shape of the temporal relationship for these complications will be of interest in increasing the understanding of pathogenesis, prognosis, study design, therapeutic effects and risk estimations.

9.12 Limitations

The main critical aspect in observational studies is to obtain similar control and intervention groups (123). In particular the two major problems are selection bias and confounders not possible to control for. The results from observational studies are particularly uncertain when studies are small and based on historical controls, when the researcher has made the clinical

follow-up, where the outcome is self-reported and where there has been no or sparse control of confounders. However, none of these aspects was the case in the present studies of modern insulins.

We controlled for several potential confounders such as age, gender, BMI, diabetes duration, duration of insulin treatment, type of diabetes, smoking, weight, daily insulin requirement, HbA1c at baseline and number of insulin doses. Another factor of interest are hypoglycaemias since several studies have reported fewer hypoglycaemias in patients treated with insulin glargine and lispro (33-35, 46, 49, 51, 52, Table 2). However, the data of hypoglycaemias in the record system were not complete and not included in the analyses.

When estimating the temporal relationship between HbA1c and retinopathy one limitation is that no estimation of the specificity was made. It is difficult to estimate a confidence interval for the temporal relation. One possibility could be to make simulations of events by means of the temporal relationship and compare with true events. A second limitation is that only a few parameters were used to reflect the temporal relationship between HbA1c and retinopathy. The reason for this was that too many parameters would make estimations more difficult and maybe not possible at all. The shape of the temporal relationship could in reality be smoother in the increasing and decreasing phases and have a less pronounced peak than the relation described. A third limitation is that extrapolations must be made when the preventive effect of a low HbA1c is estimated from diagnosis. However, there were many patients included in the DCCT with short duration of diabetes and also those with longer duration.

Another factor that should be noticed is that sustained progression of retinopathy has been used as endpoint in earlier studies (10, 60). Using the first time point for progression is more suitable in analyses e.g. since it is difficult to know if data should be censored or not when sustained progression is used and a worsening of 3 steps on the ETDRS scale occurs soon before the end of the study. It is not possible to tell if a relation would become stronger or weaker if a stricter criterion of sustained progression is used since more definite but fewer outcomes will appear.

9.13 Electronical tracking of data is important in clinical research

In the present studies of insulin analogues in clinical practice electronical tracking of data was crucial (Paper I and II). Without this tool only a small part of the patients with insulins could have been examined, possibly only patients in one small outpatient clinic instead of a large number of patients from 14-15 outpatient clinics. Furthermore the collection of HbA1c values from 12,980 diabetes patients made simulations of HbA1c variables realistic (Paper IV). There are today many retrospective studies made worldwide and data are then often collected manually which is very time-consuming and hence in many cases less efficient and competitive. Manual collection of data for quality improvement and national quality registers is also common.

In a world perspective of clinical research and the aim of a good quality controlled care for all diabetes patients the costs of data collections will be tremendous. Moreover, besides considerable cost reductions with electronical tracking much more detailed information will be gathered being of use for both research and quality control programs. Hence, we believe

that demands for possibilities of electronical tracking in all patient record systems should be an obligation.

9.14 Time-dependent effects should be evaluated in clinical trials and epidemiologic studies

Numbers needed to treat to prevent retinopathy differed as much as from 274 to 2 for different 3-year periods for the same patient population and HbA1c difference. The difference of an NNT of 2 and 274 is so great that a treatment would be regarded as extremely efficient and inefficient respectively. There are therefore many reasons for estimating time-dependent effects in trials beside the average effect. Firstly, the NNT is often used as a measure of effectiveness of a treatment among clinicians. Secondly, time-dependent effects will give an understanding if the salutary effect of a treatment will increase in clinical practice where the treatments are often used for longer periods. Thirdly, if a trial has shown results of borderline, significance an increased effect with time will guide the investigators to make a longer trial which could show a greater effect.

In epidemiologic studies it is common to search for predictors of an outcome. We found e.g. 97 epidemiologic analyses from large prospective studies or clinical trials of HbA1c and diabetic complications (Paper III, Table 1). Based on our results it could be questioned if it is rational to estimate the size of risks if not knowing if it is time-dependent. However, one problem could be that frequent measurements of outcomes and risk factors probably are needed. If such analyses are not possible in a certain study evidence from other studies of time-dependent effects could be of help in drawing conclusions. Time-dependent effects are also of importance in adjustments of analyses. If e.g. a new potential risk factor of retinopathy is studied over 3 years, HbA1c at baseline or mean HbA1c during the study will not account for the influence of previous HbA1c values. Considering the fact that several variables generally are adjusted for, each with possibly different time-dependent effects, can make the results of many epidemiologic studies today more uncertain.

The most important fact might however be that a so far unidentified risk factor for a disease that increases with longer time after exposure easily could miss being detected. It is just to remind oneself of how long time it took before impaired glycaemic control was established as a risk factor for diabetic complications although it was obvious to hypothesis that glucose control could be of importance for diabetic complications. For other diseases, so far unknown, crucial risk factors might be much less obvious to couple to the disease and it might take time until the effect of these appears making them difficult to detect. We believe from the history of glycaemic control and diabetic complications that if there is a great suspicion of a risk factor which could play an important role for the burden of disease, time-dependent effects should be evaluated and trials made with many patients over a long period of time.

9.15 Conclusions

In conclusion this thesis strongly suggests that:

- Insulin lispro and insulin glargine improve glycaemic control in clinical practice and the reductions obtained in HbA1c are clinically relevant.
- Good glycaemic control is more important than earlier believed in preventing retinopathy.
- The momentary risk of retinopathy increases dramatically with time although HbA1c is constant and the predictive ability is greater than earlier recognised.
- Patients with current good control can develop retinopathy due to earlier poor glycaemic control.
- Design of trials of HbA1c and cardiovascular disease could probably be improved if their temporal relationship is first determined.
- The importance of good glycaemic control in preventing nephropathy, neuropathy and macrovascular complications is probably substantially underestimated.
- In general in medicine time-dependent effects of treatments and risk factors should be regarded in epidemiologic and clinical trials to understand the magnitude of the effects.
- Electronical tracking of data in clinical research and quality improvement is more efficient than manual collection, extensive information is retrieved and costs are reduced substantially.

Abbreviations

A/C	Albumin creatinine
ADA	American Diabetes Association
BMI	Body mass index
CHF	Cardiac heart failure
CI	Confidence interval
CSII	Continuous subcutaneous insulin infusion
CV	Cardiovascular
CVD	Cardiovascular disease
CWS	Cotton wool spots
DCCT	Diabetes Control and Complications Trial
DECODE	Diabetes Epidemiology Collaborative Analysis of Diagnostic Criteria in Europe
DR	Diabetic retinopathy
EDIC	Epidemiology of Diabetes Interventions and Complications Study
ESRD	End stage renal disease
ETDRS	Early Treatment Diabetic Retinopathy Study
EURODIAB	European Diabetes Study
FPG	Fasting plasma glucose
GFR	Glomerular filtration rate
HbA1c	Glycated haemoglobin
HE	Hard exudates
HMA	Haemorrhages and microaneurysms
HOPE	Heart Outcomes Prevention Evaluation
HPLC	High performance liquid chromatography
IFG	Impaired fasting glucose
IGF-1	Insulin-like growth factor 1
IGT	Impaired glucose tolerance
IRMA	Intraretinal microvascular abnormalities
IQR	Interquartile range
MA	Microaneurysm
MDI	Multiple daily injections
NNT	Numbers needed to treat
NPDR	Non-proliferative diabetic retinopathy
NPH	Neutral protamine Hagedorn
NVD	New vessels on the disc
NVE	New vessels elsewhere
OGTT	Oral glucose tolerance test
PAD	Peripheral arterial disease
PDR	Proliferative diabetic retinopathy
PZI	Protamine zinc insulins
SD	Standard deviation
SDIS	Stockholm Diabetes Intervention Study
U-AER	Urinary albumine excretion rate
UKPDS	United Kingdom Prospective Diabetes Study
WESDR	Wisconsin Epidemiologic Study of Diabetic Retinopathy
WHO	World Health Organisation

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