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CLINICAL STUDIES OF GLUCOSE VARIABILITY AND HYPOGLYCEMIA IN ADULT PATIENTS WITH TYPE 1 DIABETES

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“You have brains in your head. You have feet in your shoes. You can steer yourself in any direction you choose. You're on your own.
And you know what you know. You are the guy who'll decide where to go.”

Dr. Seuss, 1904 to 1991

To Irene and David

ABSTRACT

Diabetes mellitus type 1 is a condition characterized by elevated blood glucose values due to impaired insulin production. This condition is associated with increased morbidity from micro- and macrovascular complications. Studies have shown that lowering blood glucose reduces the incidence of these complications. However, the question of whether glycemic variability is an independent risk factor for the development of microvascular complications remains. Studies **I** and **II** were retrospective observational studies performed on the same cohort of patients; it consisted of 100 patients who were randomly selected from 442 consecutive type 1 diabetic patients who attended our out-patient clinic. Standard deviation of blood glucose (SDBG) was calculated from 70 measurements over a period of 4 weeks. The onset and progression of micro- and macrovascular complications were recorded from 1990 to 2001. HbA1c values were recorded from 1985 to 2001. HbA1c variability was measured as the SD of collected HbA1c values. The prevalence of complications increased over time from 1990 to 2001. The prevalence of neuropathy increased from 29% to 47%. Statistical analysis showed that HbA_{1c} was an independent predictor of the incidence ($p = 0.004$) and the prevalence ($p = 0.01$) of nephropathy. SDBG was found to be a predictor of the prevalence of peripheral neuropathy ($p = 0.03$) and showed borderline significance for the incidence of peripheral neuropathy ($p = 0.07$). The variability of HbA1c was found to be an independent factor that contributes to the development of microvascular complications. ($p=0.019$). We conclude that the short-term variability of blood glucose may be of importance for the development of peripheral neuropathy and that nerve tissue might be particularly vulnerable and we also conclude that the variability of HbA1c may be of importance for the development of microvascular complications. Study **III** was an open, randomized, cross-over trial in 15 type 1 diabetes patients to compare glucose control assessed by a continuous glucose monitoring system (CGMS) Gold during basal insulin substitution with either a continuous subcutaneous insulin infusion (CSII) or glargine. Comparing CSII with glargine revealed that the mean blood glucose was lower with CSII, 9.11 vs. 10.31 ($p=0.002$). There was an average of 1.3 more episodes of values below 3.5 mmol/l with CSII ($p=0.053$). There were no significant differences in glucose variability measured as SDPG or the mean amplitude of glucose excursions (MAGE), although significantly longer periods of glucose values were spent within the target of 4.5-10.0 mmol/l with CSII ($p=0.034$). CSII provided superior glucose control compared with glargine, with lower mean blood glucose at longer periods of glucose values within target and a lower HbA1c with a somewhat smaller insulin dose (n.s). However, there was no significant difference with respect to glucose variability. Study **IV** was an observational study of the prevalence of severe hypoglycemia (SH) in relation to risk factors in type 1 diabetic patients over a period of 14 years, 1984 to 1998. To investigate the problem of SH, we performed a cross-sectional survey of a cohort of 178 patients registered at our out-patient clinic in 1984 to be repeated in 1998. An identical questionnaire was sent to the patients at the beginning of 1985 and 1999 respectively, regarding the problem of SH in the preceding year. The use of multiple insulin injection therapy had increased from 71% to 98% ($p<0.001$) and the daily self-monitoring of blood glucose (SMBG) from 17% to 48% ($p<0.001$), between 1984 and 1998. An increasing number of patients reported unawareness of hypoglycemia, 54% vs. 40% ($p<0.01$), and nocturnal events were more frequent, 83% vs. 76% ($p<0.05$). The prevalence of SH had increased from 17% to 27% ($p<0.05$) and there was a slight decrease in HbA1c, 7.6% to 7.4% ($p<0.05$). In spite of the more frequent use of multiple injection therapy and more frequent SMBG, the prevalence of SH increased by more than 50% over 14 years, 1984 to 1998.

LIST OF PUBLICATIONS

I. **I** Can glycaemic variability, as calculated from blood glucose self-monitoring, predict the development of complications in type 1 diabetes over a decade? Bragd J, Adamson U, Bäcklund LB, Lins PE, Moberg E, Oskarsson, P, *Diabetes Metab*. 2008 Dec; 34(6 Pt 1):612-6. Epub 2008 Sep 27.

II Can HbA1c variability contribute to the development of microvascular complications in type 1 diabetes? Bragd J, Adamson U, Lins PE, and Oskarsson P, (Manuscript)

III Basal insulin substitution with glargine or continuous subcutaneous insulin infusion in adult type 1 diabetes patients-a randomized controlled trial. Bragd J, von Döbeln A, Lins PE, Adamson U, Bergström J, Oskarsson P, *Diabetes Technol Ther*. 2010 Sep; 12(9):689-93.

IV A repeated cross-sectional survey of severe hypoglycaemia in 178 Type 1 diabetes mellitus patients performed in 1984 and 1998. Bragd J, Adamson U, Lins PE, Wredling R, Oskarsson P, *Diabet Med*. 2003 Mar; 20(3):216-9.

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LIST OF ABBREVIATIONS

ADA	=	American Diabetes Association
AGE	=	advanced glycosylation end products
AUC	=	area under the curve
b-glucose	=	blood glucose
BMI	=	body mass index
CNS	=	central nervous system
CSII	=	continuous subcutaneous insulin infusion
CGMS	=	continuous glucose monitoring system
CGS	=	continuous glucose sensor
CV	=	coefficient of variation
DCCT	=	Diabetes Control and Complication Trial
e.g.	=	exempli gratia (for example)
EV	=	emergency room visit(s)
GI	=	gastrointestinal
GV	=	glucose variability
HbA1c	=	glycosylated hemoglobin A1c
HPLC	=	high performance liquid chromatography
i.e.	=	id est (that is)
i.m.	=	intramuscular
i.v.	=	intravenous(ly)
MAGE	=	mean amplitude of glycemic excursion
MIME	=	mean indices of meal excursions
MODD	=	mean of daily differences
NDR	=	National Diabetes Registry
LBGI	=	low blood glucose index
p-glucose	=	plasma glucose
PKC	=	protein kinase C
s.c.	=	subcutaneous
SD	=	standard deviation
SDBG	=	standard deviation of blood glucose
SDPG	=	standard deviation of plasma glucose
SDIS	=	Stockholm Diabetes Intervention Study
SH	=	severe hypoglycemia
SEM	=	standard error of mean
SMBG	=	self-monitoring of blood glucose
SMPG	=	self-monitoring of plasma glucose
VAS	=	visual analog scale

1 INTRODUCTION

In spite of the fact that treatment with insulin for patients with type 1 diabetes has been available since the early 1920s and that a relationship between hyperglycemia and the progression of diabetic micro-angiopathy was demonstrated in the 1970s (Pirart, Lauvaux et al. 1978), it was not until the 1990s that proof of a protective effect by good glycemic control on long-term diabetic complications was obtained (Reichard, Berglund et al. 1991),(DCCT 1993). The risk of the onset/progression of angiopathy was thus reduced by 50-75% in intensively treated patients over a time period of 8-10 years in the Diabetes Control and Complication Trial (DCCT) study. Notably, this effect was demonstrable for retinopathy, nephropathy and also neuropathy.
The results of the DCCT study were interpreted as indicating that, regardless of the level of HbA1c at entry to the study, a similar reduction was required to obtain a 50% reduction in the risk in the intensively treated group of patients (Nathan, Cleary et al. 2005). Consequently, the most beneficial effects on diabetic complications were registered among patients with the highest initial levels of HbA1c being at the highest risk. More recent analyses have indicated that intensive insulin therapy in itself may reduce the risk of complications to some extent, beyond its effect on HbA1c, and this has raised the issue of whether the variability in glycemia may play a role in the pathophysiological process underlying the development of micro-angiopathy (Service and O'Brien 2001).

In the DCCT study and in the SDIS trial (Reichard, Berglund et al. 1991), it was observed that the incidence of severe hypoglycemia increased markedly when intensive treatment with the object of attaining near-normoglycemia was instituted. Hypoglycemia is a common acute complication of insulin therapy in subjects with type 1 diabetes. Severe events of hypoglycemia occur annually among approximately 30% of these patients and hypoglycemic coma is believed to affect 10% a year. The risk of severe hypoglycemia, defined as an event which requires third-party assistance, is known to be particularly high in subjects who display the phenomenon of unawareness of hypoglycemia, i.e. who fail to recognize impending low blood glucose. To summarize, problems related to hypoglycemia are most likely underestimated in clinical practice and hypoglycemia remains a major therapeutic problem causing considerable fear among patients and relatives (Anderbro, Amsberg et al. 2010).

The self-monitoring of blood glucose (SMBG) became widely available for diabetic patients in Sweden in 1981, when it was provided free of charge. A novel instrument to assist patients to optimize insulin therapy and to help detect impending hypoglycemia was thus made widely available. Furthermore, in the 1980s, portable insulin infusion pumps for continuous subcutaneous insulin infusion were introduced on the market, along with the implementation of multiple injection therapy as standard care. At a later stage, short- and long-acting insulin analogs were introduced to help improve these treatment regimens. On the one hand, many patients with type 1 diabetes witness marked improvements in their quality of life with multiple injection therapy, while, on the other, recent data from the Swedish National Diabetes Registry (NDR 2009) demonstrate that only a minority of patients obtain the level of glycemic control which has been put forward as a general target by the National Board of Health and Welfare (Socialstyrelsen Riktlinjer 2009). Over time, it has been increasingly acknowledged that SMBG on a 4-7 times daily basis fails to disclose many hypoglycemic events, in particular at night, and that the optimization of basal insulin substitution with long-acting analogs is unable to mimic the

diurnal variation in plasma insulin seen in non-diabetics. Portable glucose sensors may provide an adjuvant to insulin therapy in this respect.

2 BACKGROUND

2.1 PATHOGENESIS OF DIABETIC MICROANGIOPATHY

The pathophysiology of diabetic micro-angiopathy, i.e. retinopathy, nephropathy and neuropathy, is multifactorial, but long-term hyperglycemia appears to play a crucial role (Pirart, Lauvaux et al. 1978). Individual differences in the susceptibility to respond to the metabolic derangements suggest that genetic and environmental factors may also be of importance. Changes in the microvascular wall, circulating blood components and hemodynamic control are also regarded as important in this context (Parving, Viberti et al. 1983), (Zatz and Brenner 1986), (Deckert, Feldt-Rasmussen et al. 1989).

2.1.1 Metabolic Alterations

Hyperglycemia appears to be toxic for endothelial cells (Lorenzi, Toledo et al. 1987), but its effect can be difficult to distinguish from those of concomitant biochemical processes. The increased metabolism of glucose by the polyol pathway results in the intracellular accumulation of sorbitol and it was previously anticipated that this caused an osmotic disturbance subsequently generating cell damage. More recent observations suggest, however, that concentrations of sorbitol obtained in association with chronic hyperglycemia are too low to exert osmotic effects at cellular level. In fact, the increased metabolism of glucose via the sorbitol pathway may induce biochemical alterations, which induce increased oxidative stress and cell damage (Chung, Ho et al. 2003).
Clinical trials with aldose-reductase inhibitors have, however, failed to document beneficial effects on retinopathy. The formation of non-enzymatic advanced glycosylation end products (AGE) is enhanced in diabetes and results in accumulation in blood vessels and glomeruli (Mullarkey C.J 1994). AGE may modify circulating plasma proteins binding to endothelial cells, thereby causing cellular damage. Furthermore, the production of protein kinase C (PKC) is increased in diabetes and it is assumed that this may affect factors such as blood flow, capillary permeability, angiogenesis and even inflammatory responses (Geraldes and King 2010).

2.1.2 Hemodynamic Changes

Hemodynamic changes to consider in diabetic patients are increasing blood flow, capillary pressure and vascular permeability (Tooke 1989, Parving et al. 1983, Zatz et al. 1986, Bollinger et al. 1982). All these functional changes appear to be linked to organ failure in susceptible tissues causing clinically relevant conditions, e.g. visual loss and renal failure. Although the relationship between functional changes and changes in the vasculature remains unclear, they may have some pathogenic mechanisms in common (Tooke et al. 1994). Increased basal blood flow early in diabetic life has been demonstrated in the kidney (Ditzel et al. 1972), retina (Kohner et al. 1975) and limbs (Gundersen 1974) and the increased microvascular leakage in diabetic patients may be due to an overperfused vascular bed and raised capillary blood pressure (Rayman et al. 1985). When diabetes progresses and clinical complications become evident, maximum tissue perfusion and vascular autoregulation are generally impaired in many organs as a result of structural abnormalities of the vascular tree (Faris et al. 1982, Newrick et al. 1988).

2.1.3 Disturbances in Hemostasis

Abnormalities in blood viscosity have been described in diabetes, partly as a result of increased levels of plasma fibrinogen, the increased generation of thrombin and the activation of platelets

(Jokl et al. 1997, Jax et al. 2009). The increased production of PKC, well described in diabetes, stimulates the production of plasminogen-activator-inhibitor-1, which inhibits the activation of plasminogen, leading to reduced fibrinolytic capacity (Geraldes et al. 2010). Most studies of fibrinolytic functions in diabetes have been performed in type 2 diabetic patients. In contrast, however, there is at least one study which actually found that the fibrinolytic capacity was increased in subjects with type 1 diabetes (Walmsley et al. 1991).

2.2 MONITORING GLYCEMIC CONTROL

2.2.1 Historical Perspectives

In the years immediately before the introduction of insulin treatment, it was demonstrated that the blood glucose concentration of healthy individuals was fairly constant under average conditions of diet and exercise. It was thus established by Rolly and Oppermann in 1913 that, in normal subjects, the fasting plasma glucose concentration ranged between 78 and 107 mg /dl and that it was somewhat lower for whole blood including corpuscles. During the years following the introduction of insulin, the methods of blood glucose determination were further developed with the aim of obtaining less time-consuming methods, but it was found in parallel that wide differences in blood sugar values of up to 30 mg/dl were observed with the methods that were applied. In the early 1930s, methods using zinc or ferric hydroxide, whereby the red cells remained intact and the filtrate was thus unaffected by non-fermentable, non-glucose substances were regarded as giving true glucose values for human blood (Herbert and Bourne 1930). One of these methods was further modified in the 1940s when an arsenomolybdate color reagent was substituted for the measurement of reduced copper (Nelson 1944). In the 1950s, enzymatic glucose-specific methods were introduced of which the glucose oxidase method has become the most widely used in current practice (Raabo and Terkildsen 1960). In this method, the determination of glucose is based on two reactions and the intensity of the color that forms is proportional to the glucose concentration in the specimen. The first report of a biosensor for glucose in which the enzyme reaction takes place in close contact with a transducer was presented in the early 1960s (Clark and Eyzaguirre 1962). The first reagent strip was introduced on the market in 1964 and soon thereafter the first reflectance meter for reading the results became available. Finally, in 1981, the self-monitoring of blood glucose became available on a larger scale when the test strips became free of charge in Sweden.

2.2.2 SMBG (Self-monitoring of Blood Glucose) in Clinical Practice

Since it was difficult to quantify the concentration of glucose in urine or blood until the 1960s, the diagnosis was mainly based on clinical symptoms such as thirst and increased volumes of urine. The first tool which came into use in clinical practice for glucose control was urine strips. The patient was asked to bring a urine sample and it was subsequently tested with a strip; the darker the color, the higher the concentration of glucose. Urine glucose was, however, in itself not sufficient to make a reliable assessment of the patient’s glucose levels.

With the introduction of portable glucose home meters for daily glucose control, it was possible for the first time with adequate safety to optimize insulin doses and to check the patient’s metabolic control.

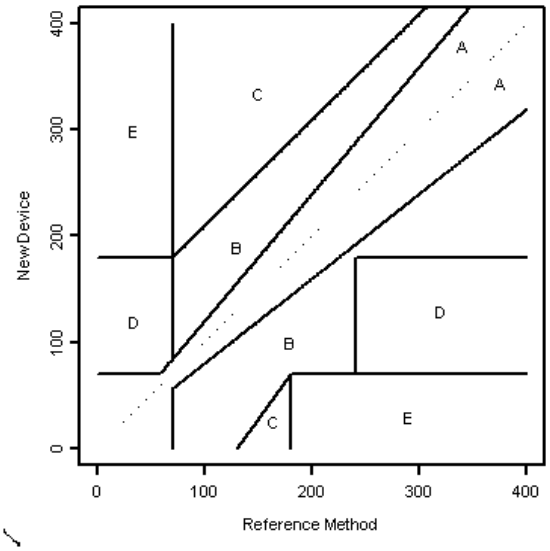
SMBG is designed to supply patients and health professionals with reliable information on the patient’s glycemic control. It is recommended that SMBG should be carried out three or more times daily for patients using multiple insulin injections or insulin pump therapy (ADA Standards of medical care in diabetes 2011). In a multicenter study, Schütt et al. in 2006 describe the relationship between the number of SMBG and HbA1c lowering. In this material, every extra SMBG test resulted in a reduction in HbA1c of 0.32% in type 1 diabetic patients with MDI

or CSII (Schutt, Kern et al. 2006). The SMBG values before dinner and in the evening correlate better with HbA1c than fasting values or values before lunch (DCCT 1993).

It is important that blood glucose meters have adequate safety and reliability in order satisfactorily to measure glucose levels. Unfortunately, different values will be obtained if various meters are tested at the same time. In the area of high blood glucose, this has a minor clinical impact, but, in the lower range of blood glucose, it could be of major importance. Over the years, manufacturers have improved glucose meters considerably. Other pitfalls when testing blood glucose are more patient related – for example, after eating a sweet fruit and getting juice on the fingers, not enough blood on the test strip, cold fingers and so on. However, more facts to consider are the change from blood glucose to plasma glucose. Glucose values assessed as plasma glucose are 11% higher than blood glucose values.

When measuring glucose, accuracy can be defined by comparison with a standard method, isotope dilution mass spectrometry. However, it is rare to determine the technical accuracy of glucose meters against the standard isotope dilution mass spectrometry method. Normally, the accuracy of a glucose meter is assessed by comparison with the routine method used in the clinical laboratory. To assess the clinical accuracy of a glucose meter, one method is to use the error grid analysis.

Clark's error grid for estimating the accuracy of SMBG.



The accuracy and error of error grid analysis are categorized into zones of accuracy.
Zone A, clinically accurate (leading to correct, safe treatment decisions); zone B, benign errors (blood glucose values outside present precision tolerances (usually within 20% of reference) but probably not resulting in deleterious decision making); zone C, overcorrection errors (result outside target range when reference is within target range, leading to treatment decisions that could result in blood glucose values outside the target range); zone D, failure to detect (high or low blood glucose) errors (resulting in failure to treat either low or high blood glucose results appropriately); and zone E, erroneous errors (blood glucose values directly opposite to reference values leading to treatment decisions opposite to those needed). (Clarke, Cox et al. 1987; Clarke, Anderson et al. 2005).

2.2.3 How to Assess Glucose Variability

Several factors are of importance in validating a patient's glucose variability. It should be a true measure of blood glucose fluctuations from a statistical point of view. Constant high or low blood glucose levels should not lead to a high variability score, but extremes must also be taken into consideration when assessing the patient. Another important factor is the time sequence, i.e. the intra-day variation compared with variations over longer periods of time, weeks or months. In recent years, the continuous glucose monitoring system has become a new and important method for assessing glycemic variation. The CGM system gives the clinician an opportunity to assess the variability during both the day and night.

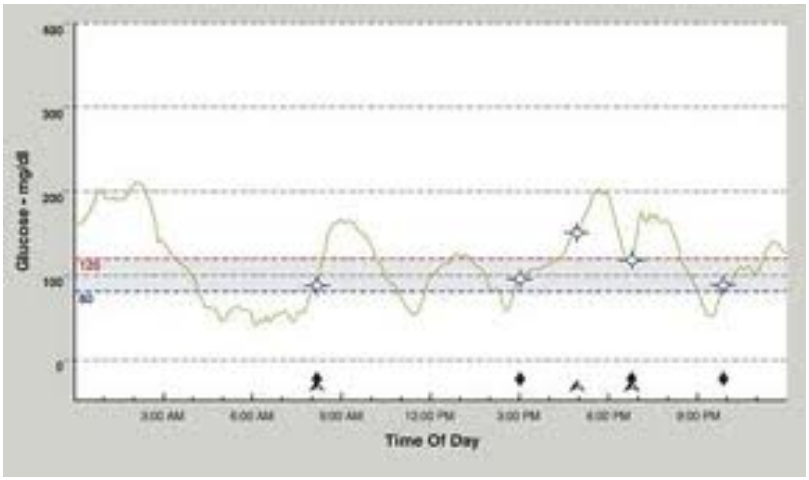
Specific mathematical methods have been developed to evaluate glycemic variations. They include the M value (Schlichtkrull, Munck et al. 1965), the mean amplitude of glucose excursion (MAGE) (Service, Molnar et al. 1970), day-to-day glycemic variations or excursions (MODD) (Molnar, Taylor et al. 1972), meal-related glycemic excursions (MIME) and the low blood glucose index (LBGI) (Selam 2000). The M value measures the deviation from a given standard and takes special consideration of hypoglycemic values. Since the M value does not measure the true glucose variability, constantly high as well as low glucose levels will result in a high M value. The MAGE, on the other hand, was originally calculated from continuous blood glucose monitoring over 48 h and measures glucose variations exceeding one standard deviation (SD) of all values. However, since the SD differs from patient to patient, the level that is regarded as significant will differ. On the other hand, MAGE gives more weight to clinically important extremes (Service, O'Brien et al. 1987). The MODD is an index of day-to-day variability and measures the absolute difference between glucose values recorded at corresponding times of the day on two consecutive days. MIME measures mean indices of meal excursions and the LGBI measures the risk of severe hypoglycemia.

All these methods are, however, relatively laborious. In contrast, the standard deviation of blood glucose (SDBG) is an easily available glucose index, which has been used extensively to identify significant glycemic characteristics in clinical trials during the past decade and virtually all current glycemic control software features calculations of standard deviation (SD).

One limitation of SD that has been put forward is that it is calculated from all measurements, including both minor and major variations, and therefore gives less weight to the extremes and the sequence of the values.

In a previous study, we have shown that SDBG measurements are highly reproducible ($r = 0.90$, $p < 0.0001$) when assessed over a period of 12 months (Moberg, Kollind et al. 1993). Studies have also documented that SDBG is not related to the HbA_{1c} level (Derr, Garrett et al. 2003). Since SDBG is easily obtained, it has been recommended (Monnier, Colette et al. 2008) as a measurement of glucose variability. A study by Baghurst et al. revealed that, in patients with type 1 diabetes, glucose variability (GV), as measured by SD or CONGA4, becomes unreliable if observations are more than 2-4 h apart and estimates of MAGE become unreliable if glucose measurements are more than 1 h apart.(Baghurst, Rodbard et al. 2010).

CGMS MiniMed Gold curve



2.2.4 Continuous Glucose Monitoring

The development of CGMS provides an instrument with which an analysis of glucose profiles over 24 hours can be performed. The obvious advantage of CGMS over the self-monitoring of plasma glucose (SMPG) is the opportunity to monitor nocturnal profiles of glucose and also the true variability of the glucose profile. The first device to be approved by the US Food and Drug Administration (FDA) and to be made available for clinical use was the Continuous Monitoring System (CGMS) (Medtronic Minimed, Northridge, CA). This is a sensor system designed continuously to monitor interstitial fluid glucose. The sensor is a microelectrode that is inserted in the subcutaneous tissue and generates an electronic signal. The strength of the electronic signal is proportional to the amount of glucose in the surrounding interstitial fluid. It measures glucose levels between 2.2 mmol/l and 22.2 mmol/l. The system generates 288 readings every 24 hours. The system is “blinded” to the patient and can therefore only be used as a retrospective tool for assessing the glucose profile of the patient. The system has to be calibrated 3-4 times every 24 hours. The system has been promoted and used as a tool to evaluate overnight glucose monitoring to detect nighttime hypoglycemia (Chase, Kim et al. 2001), (Boland, Monsod et al. 2001). In these studies, however, there were no confirmatory meter readings. A study from 2002 performed by McGowan et al. compared simultaneously measured glucose values with a glucose analyzer, the Accu-Check Advantage meter, and a CGMS when assessing nighttime hypoglycemia. The authors drew the conclusion that CGMS reports of asymptomatic nighttime hypoglycemia may be spurious and should be interpreted with caution in patients with tightly controlled diabetes (McGowan, Thomas et al. 2002). Since then, a second-generation device has been developed by Medtronic, the CGMS Gold. In our study group, we have used the CGMS Gold as a tool to evaluate glucose variability and other measurements of glucose control.(Bragd, von Dobeln et al. 2010). We will also use this device in a future study, where we will assess glucose variability in a randomized, placebo-controlled, cross-over study with the drug domperidone.

CGMS MiniMed **Gold**

In recent years, devices for “real-time” continuous glucose monitoring have been introduced as a tool to achieve improved overall glycemic control. Several CGSs have the ability to measure and transmit real-time blood glucose values to the patient and/or to warn of impending hypo/hyperglycemia with audible alarms. “Real-time” CGSs have been shown to improve HbA1c in patients with type 1 diabetes. (Deiss, Bolinder et al. 2006),(Beck, Hirsch et al. 2009), (Tamborlane, Beck et al. 2008), (O'Connell, Donath et al. 2009). In a study performed by Battelino et al. published in 2011, it was shown that “real-time” continuous glucose monitoring was associated with reduced time spent in hypoglycemia and a concomitant decrease in HbA1c in children and adults with type 1 diabetes under very tight control. (Battelino, Phillip et al. 2011). In this study, they used the FreeStyle Navigator (Abbott Diabetes Care). Like the CGMS (Medtronic Minimed, Northridge, CA), the FreeStyle system also measures the glucose in interstitial fluid. In clinical practice, recommendations from the American Diabetes Association in Standards of Medical Care suggested that continuous glucose monitoring is especially useful in patients with hypoglycemia unawareness and/or frequent episodes of hypoglycemia. In 2011 in Sweden, the governmental agency (TLV) 2 decided that it would not reimburse “real-time” continuous glucose monitoring devices and this decision has stirred up strong feelings among patients and an appeal against the decision has been made in a court of law. At the moment, the decision is on hold. However, the question of whether the patients who would benefit from these devices will be able to get them in the future still arises.

2.3 GLYCOSYLATED HEMOGLOBIN (HbA1c)

2.3.1 Glycosylation

Since the late 1970s, Hba1c has been used indirectly to measure the blood glucose levels of diabetic patients. The sample reflects the mean blood glucose level in the last 4 to 8 weeks. When measuring glycosylated hemoglobin, it is the proportion of hemoglobin molecules that are bound to glucose that is measured. Glucose binds irreversibly to the hemoglobin molecule in a direct relationship with the blood glucose level. Increased average blood glucose leads to a higher HbA1c. Because the binding is irreversible, glucose is connected throughout the life span of the erythrocyte, approximately 120 days. This means that conditions with increased circulation of erythrocytes will result in false low values of HbA1c. Examples of these conditions include thalassemia, hemolytic anemia and bleeding resulting in blood transfusion. False high values of HbA1c are less common, but, if the erythropoiesis stops and there are mainly older erythrocytes in the circulation, which are exposed for longer to glucose, HbA1c might rise (Landin-Olsson, Jeppsson et al. 2010).
The hemoglobin molecule consists of two alpha chains and two beta chains. Glucose binds to all the amino acid, valine, in the beta chain. Glucose can also bind to either valine in an alpha chain or to the amino acid, lysine. The glycosylation process starts with the formation of a Schiff base, when the aldehyde group of a glucose molecule binds with the amino group of a valine molecule. This is a double bond between carbon atoms in the glucose and the nitrogen atom of valine. An Amadori product then forms in which the Schiff base changes its structure. A hydrogen atom in the hydroxyl group moves and binds to the nitrogen atom. Eventually, an irreversible binding is created via oxidation and the glycosylation process is finished. It is the same reaction when advanced glycosylation end products (AGE) are produced. In other words, there are three steps in the complete reaction. The first two steps are reversible, but the last step is irreversible. The whole process is called a Maillard reaction.

2.3.2 HbA1c in Clinical Practice

Initially, it was thought that the HbA1c reflected mean glucose over some 2-3 months (Nathan, Singer et al. 1984). HbA1c is currently thought to reflect mean blood glucose during approximately 4-8 weeks (Berne 2010). How often should HbA1c be tested in patients with type 1 diabetes? The recommendations from the USA and ADA for testing HbA1c are as follows. 1. Perform the HbA1c test at least twice a year in patients who are meeting treatment goals and who have stable glycemic control. 2. Perform the HbA1c test quarterly in patients whose therapy has changed or who are not meeting glycemic goals. 3. The use of points-of-care testing for HbA1c allows for timely decisions on therapy changes, when needed (ADA Standards of medical care in diabetes) (2011). In clinical practice, this means that about 2-4 HbA1c values are recorded by the patients annually when visiting the out-patient clinic at the hospital.

How are things in a Swedish population? At our out-patient clinic at Danderyd Hospital, the patients normally visit the clinic 2-4 times a year, resulting in 2-4 HbA1c tests. Due to the lack of resources in recent years, a standard patient is not able to visit the clinic more than twice a year. We do, however, have an opportunity to collect HbA1c either from the lab where patients go to take the test or when the patient sends a blood sample to the lab by post.

HbA1c can be measured using several methods. There are three different standards in different parts of the world when measuring HbA1c. 1: DCCT (the Diabetes Control and Complications Trial) standard that is managed through the organization known as the National Glycoprotein Standardization Program (NGSP), 2: The Japanese Diabetes Society (JDS/JSCC) standardization and, finally, the Swedish Mono-S method. The NGSP standard is about 0.9% higher than Mono-S. The reference for Mono-S is 3.6-5.2%. Because there have been three standards around the world for HbA1c, it has been difficult to compare studies from different

countries. For years, medical scientists have tried to agree on a standard for the whole world. This has now been successful and, since 1 October 2010, a general standard has been agreed for the whole world. The organization known as the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) has succeeded in obtaining this agreement (2007; Nordin 2008; Hanas and John 2010; Little, Rohlfing et al. 2011). The current quality goal for HbA1c among Swedish laboratories is a maximum bias of 0.2% (average deviation in EQUALIS surveys over 6 months) and that individual EQUALIS results should deviate less than 0.4% HbA1c units from the assigned target value (Hanas and John 2010).

A table for the conversion of HbA1c to the new IFCC standard now follows.

B–HbA_{1c} Mono S (%)	B–HbA_{1c} IFCC (mmol/mol)	B–HbA_{1c} NGSP (%)	B–HbA_{1c} Mono S (%)	B–HbA_{1c} IFCC (mmol/mol)	B–HbA_{1c} NGSP (%)
2,5	16	3,6	9,0	83	9,8
3,0	21	4,1	9,5	89	10,3
3,5	26	4,5	10,0	94	10,7
4,0	31	5,0	10,5	99	11,2
4,5	36	5,5	11,0	104	11,7
5,0	42	6,0	11,5	110	12,2
5,5	47	6,4	12,0	115	12,7
6,0	52	6,9	12,5	120	13,1
6,5	57	7,4	13,0	125	13,6
7,0	63	7,9	13,5	130	14,1
7,5	68	8,4	14,0	136	14,6
8,0	73	8,8	14,5	141	15,0
8.5	78	9.3	15.0	146	15.5

2.4 INSULIN TREATMENT IN PATIENTS WITH TYPE 1 DIABETES

2.4.1 Historical Aspects

Insulin was discovered by Fredrik Banting, Charles Best and coworkers in 1921. This is regarded as one of the most important discoveries in medical history and the researchers were shortly thereafter awarded the Nobel Prize in Medicine for developing this life-saving treatment for patients with diabetes. The first patient to receive insulin was the fourteen-year-old boy Leonard Thompson. He was injected with insulin on 11 January 1922. It was an immediate success and the news traveled around the world and the pharmaceutical industry started manufacturing insulin preparations so that this treatment became available to a great many patients around the globe. Initially, the insulin was manufactured through extraction from bovine pancreases and insulin was provided as either a powder or a tablet to be mixed with water to form a solution to be injected subcutaneously. In the beginning, these solutions were relatively contaminated. However, through crystallization, the manufacturers were able to improve the quality of their products. In the 1960s, the purification process was further improved by the introduction of chromatography. One downside of water-soluble insulin is that its effect is of relatively short duration. The first available insulin with prolonged effect duration was protamin-zinc insulin and the prolonged effect was obtained by slower absorption due to the addition of zinc and protamin to form insulin complexes which had been too large to absorb prior to degradation. The hybrid-DNA process has led to refined products and, as a result, either human insulin or modified insulin analogs are now widely used for the treatment of type 1 diabetes.

2.4.2 Physiological and Pharmacological Aspects

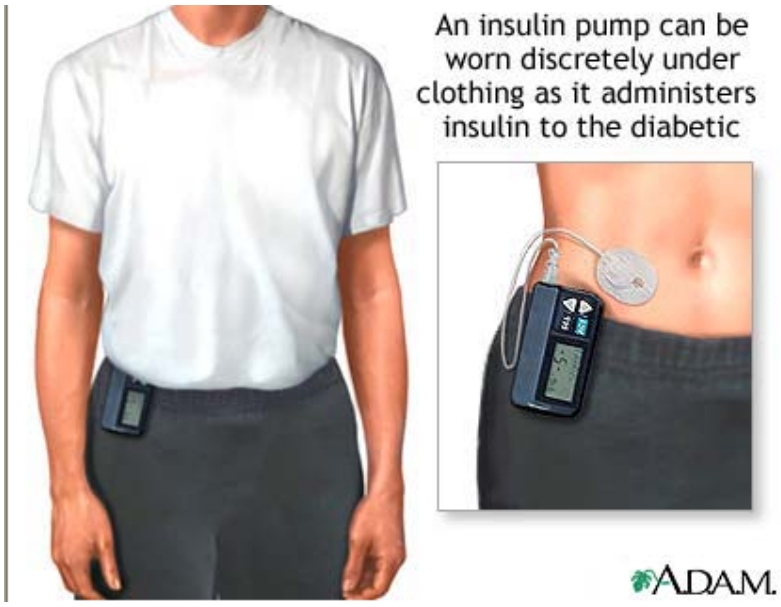
In normal humans, blood glucose is maintained within a narrow range by feedback control mechanisms in which insulin is secreted into the portal vein on a minute-by-minute basis in response to variations in the plasma levels of glucose, nutrients and a series of regulatory factors including gastrointestinal hormones, catecholamines and so on. In patients with type 1 diabetes, treatment with subcutaneously injected or infused insulin gives a poor imitation of the physiological secretion of insulin due to slow and variable insulin absorption rates and a lack of feedback control. Periods of insulin deficiency as well as peripheral hyperinsulinemia are therefore likely to occur with this therapy, with the result that the diabetic patient may experience unpredicted swings in blood glucose. It has been estimated that conventional methods of injecting insulin subcutaneously may explain up to 80% of these unpredicted glycemic swings, since the intra-individual day-to-day variation in insulin absorption rates may be as high as 35% (Lauritzen, Faber et al. 1979), (Binder, Lauritzen et al. 1984). Using short-acting insulin preparations only, e.g. during continuous subcutaneous insulin infusion therapy (CSII), only small subcutaneous depots of insulin are established and this may help to reduce this source of erratic insulin absorption and subsequent glycemic instability (Lauritzen, Pramming et al. 1983).

2.4.3 Multiple Injection Therapy

In the late 1970s, insulin treatment by three daily injections of short-acting insulin before main meals, combined with one daily injection of long-acting insulin, was advocated (Eschwege, Guyot-Argenton et al. 1976). When SMBG became free of charge in Sweden in 1981, this treatment regimen was integrated with regular SMBG to constitute “standard care”. It soon became evident that this treatment regimen was very well accepted by diabetes patients, as it offered more freedom with respect to the timing of meals, exercise and so on. Assessments of quality of life among these patients provided convincing evidence to support the concept that these regimens should be put forward as “standard care”.

2.4.4 Insulin Pump Treatment (continuous subcutaneous insulin infusion, CSII)

The first report on CSII appeared in the *British Medical Journal* in 1978, when Pickup and coworkers from Guy’s Hospital in London presented their experience of a novel technique to infuse insulin continuously into subcutaneous tissue using an external motor-driven syringe pump (Pickup, Keen et al. 1978). The new device provided basal insulin delivery and requirements during meals. The use of insulin pumps in Sweden increased rapidly during the 1980s and 1990s and it is currently estimated that approximately 20% of type 1 diabetes patients are using insulin pumps on a regular basis. It is generally held that CSII, in comparison with multiple injection therapy, results in a modest yet worthwhile improvement in glycated hemoglobin, but its main value may be in reducing other problems such as hypoglycemia (Bode, Steed et al. 1996), the dawn phenomenon and improving quality of life by allowing greater flexibility of lifestyle (Colquitt, Green et al. 2004). An increased risk of ketoacidosis during CSII has been reported and it must certainly be taken into consideration in clinical practice.



Insulin pen compared with an old syringe.

2.5 HYPOGLYCEMIA AND TYPE 1 DIABETES

2.5.1 Biochemical Definitions of Hypoglycemia

When considering the frequency of hypoglycemia in clinical practice, criteria for what constitutes a hypoglycemic event are required. The problem is that, in research into the epidemiology of hypoglycemia, no consensus on common definitions has been employed. The use of a biochemical definition of hypoglycemia, addressing a given blood glucose concentration below which hypoglycemia would be deemed to occur, would seem justified. Unfortunately, and for a number of reasons, it is not possible to define such a precise criterion for hypoglycemia (Service 1995).

Along with a declining blood glucose concentration, a series of events take place at glycemic thresholds that are individually set and may vary depending on the preceding or prevailing glycemic control. At an arterialized blood glucose value of approximately 3.8 mmol/l, counter-regulation is activated and the impairment of cognitive functions then follows at a level between 3.2-2.6 mmol/l. In clinical practice, however, capillary or venous sampling is used and these levels are lower than the arterialized ones (Liu, Adamson et al. 1992). In their clinical practice recommendations, the ADA propose a blood glucose value of 3.9 mmol/l to represent hypoglycemia (ADA Workgroup on Hypoglycemia), while Diabetes UK gives recommendations to patients with diabetes to ensure that their blood glucose does not fall below 4 mmol/l (Holt 2011).

2.5.2 Clinical Definitions of Hypoglycemia

In the absence of a biochemical definition of hypoglycemia, clinical definitions based on symptomatology have been adopted. Since symptoms of hypoglycemia are not specific evidence of biochemical hypoglycemia, they have to be supportive. For this reason, hypoglycemia is usually defined as an episode in which typical symptoms occur and the symptoms are reversed by treatment to raise blood glucose. In this way, *asymptomatic hypoglycemia* is defined as low blood glucose identified at routine testing with no associated symptoms, *mild symptomatic hypoglycemia* as symptoms suggestive of hypoglycemia successfully treated by the patient alone and *severe hypoglycemia* as an event where assistance from a third party was required to effect treatment. Finally, the term *profound hypoglycemia* is used to describe an event associated with permanent neurological deficits or death.

2.5.3 Frequency of Hypoglycemia

Most episodes of hypoglycemia occur at home, at work or during leisure activities and the subsequent recall of these episodes by patients is generally poor, particularly with regard to mild hypoglycemia. Against this background, it is obvious that data obtained prospectively have a strong advantage over those reported in retrospective studies. Moreover, the presence of an impaired awareness of hypoglycemia, the quality of glycemic control and the nature of the population of patients that is studied have to be kept in mind when comparing figures presented by different research groups.

It is worth noting, for example, that mild hypoglycemia frequencies between 8 (Leckie, Graham et al. 2005) and 160 (Janssen, Snoek et al. 2000) episodes per patient and year have been presented.

Severe hypoglycemia is generally held to be a more robust end-point than mild hypoglycemia and, against this background, retrospective recall is felt to be much more reliable. For example, Pedersen-Bjergaard reported that 90% of subjects correctly recalled their experience of severe hypoglycemia from the preceding year (Pedersen-Bjergaard, Pramming et al. 2004). Frequencies of severe hypoglycemia, defined as above, in adults with type 1 diabetes, reported between 1991 and 2005, vary from 0.98 (Leckie, Graham et al. 2005) to 1.6 (MacLeod, Hepburn et al. 1993) episodes/patient/year, while the corresponding figures for proportion affected vary from 3% (Pramming, Thorsteinsson et al. 1991) to 41% (ter Braak, Appelman et al. 2000).

2.5.4 Causes of Hypoglycemia

Hypoglycemia in people with type 1 diabetes results from an imbalance between insulin-mediated glucose efflux from the blood stream and the amount entering the circulation from the liver or from ingested carbohydrate. In principle, the causes of hypoglycemia can be attributed to inappropriate insulin injection, inadequate exogenous carbohydrate intake, increased

carbohydrate utilization, increased insulin sensitivity and a reduction in endogenous glucose production or insulin clearance (Cryer, Davis et al. 2003). In clinical practice, these causes correspond to the excessive dosing or inappropriate timing of insulin, missed meals, exercise, nocturnal rest, excessive alcohol consumption and renal failure.

2.5.5 Impaired Awareness of Hypoglycemia

Back in the early era of insulin therapy, it was observed that dangerous hypoglycemia may occur without warning symptoms (Joslin 1924).
The mechanisms underlying this impaired awareness or unawareness of hypoglycemia are not known and may be multifactorial. In principle, chronic exposure to low blood glucose will evoke an *adaptation of the central nervous system* to hypoglycemia which is clinically fairly evident in diabetic patients under strict glycemic control (Boyle, Kempers et al. 1995) and which is frequently observed in insulinoma patients. (Mitrakou, Fanelli et al. 1993). Notably, when impaired awareness of hypoglycemia results from strict metabolic control in type 1 diabetic patients, this can usually be reversed by the scrupulous avoidance of hypoglycemia based on frequent blood glucose monitoring.

Other mechanisms that may cause impaired awareness of hypoglycemia and have been disclosed are CNS glucoregulatory failure due either to *counter-regulatory deficiency* or to hypoglycemia-associated *autonomic failure*(Cryer 1992), as well as the dysfunction of the peripheral nervous system, clinically known as peripheral *autonomic neuropathy* (Hoeldtke, Boden et al. 1982). Reduced *peripheral adrenoceptor sensitivity* has also been considered in this context.

For many years, clinicians have recognized that patients with a long duration of diabetes require lower blood glucose to provoke a symptomatic response. In 1941, Lawrence wrote that “as years of insulin life go on, sometimes only after 5-10 years I find it almost the rule that the type of insulin reaction changes, the premonitory autonomic symptoms are missed out and the patient proceeds directly to the more serious manifestations affecting the central nervous system”. Clearly, the prevalence of impaired awareness of hypoglycemia or hypoglycemia unawareness increases with the duration of insulin-treated diabetes (Hepburn, Patrick et al. 1990) so that almost 50% of patients experience hypoglycemia without warning symptoms after 25 years of treatment (Pramming, Thorsteinsson et al. 1991) and hypoglycemia unawareness could therefore be recognized as an acquired abnormality associated with insulin therapy.

2.5.6 Risk Factors for Severe Hypoglycemia

It is a common clinical observation that it is often not possible to identify the precipitating cause of an episode of hypoglycemia, even though there was third-party involvement in its treatment. In some studies, figures as high as 40% have been reported (Potter et al. 1982) to illustrate this problem. It has therefore been increasingly recognized that it is necessary to look beyond conventional precipitating factors and to consider other phenomena of possible importance. Epidemiological studies and randomized controlled trials have demonstrated that *intensive therapy* (DCCT (1997), (Reichard, Toomingas et al. 1994), *low HbA1c* (EURODIAB (1994), (Bott, Bott et al. 1997), (Pedersen-Bjergaard, Pramming et al. 2004), *a previous episode* (MacLeod, Hepburn et al. 1993), (Bott, Bott et al. 1997), (Gold, Frier et al. 1997), (Muhlhauser, Overmann et al. 1998), *C-peptide negativity* (Muhlhauser, Overmann et al. 1998), (Pedersen-Bjergaard, Agerholm-Larsen et al. 2001), *age* (Gold, Frier et al. 1997), (Leese, Wang et al. 2003), *insulin dose* (ter Braak, Appelman et al. 2000) and *ACE activity* (Pedersen-Bjergaard, Agerholm-Larsen et al. 2001), (Pedersen-Bjergaard, Agerholm-Larsen et al. 2003) are risk

factors to be considered, along with *impaired awareness* and *duration of diabetes*, documented in a number of studies in recent years.

Sleep impairs both the physiological and behavioral responses to hypoglycemia and it is generally held that *nocturnal episodes* of hypoglycemia often fulfill the criteria of a severe event. Currently, in clinical practice, the development of strategies to reduce the risk of nocturnal hypoglycemia is clearly an area on which to focus.

The incidence of severe hypoglycemia was reported as being higher in patients with *impaired renal function* (Muhlhauser, Toth et al. 1991), (Pedersen-Bjergaard, Pramming et al. 2004), (ter Braak, Appelman et al. 2000). This association could, however, be confounded by many factors, such as acquired hypoglycemia syndromes and concomitant drug therapy, and a similar comment has been made about the recent suggestion that *smoking* is a risk factor for severe hypoglycemia (Pedersen-Bjergaard, Pramming et al. 2004).

Socioeconomic status (Muhlhauser, Overmann et al. 1998), *low mood* (Gonder-Frederick, Clarke et al. 1997) and an individual's attitude towards carrying *a supply of carbohydrate* (Bott, Bott et al. 1997) have also been put forward as being of importance, in line with recent data relating to ACE activity, which address the issue of whether a *genetic susceptibility* to hypoglycemia may exist (Pedersen-Bjergaard, Pramming et al. 2004).

3 AIMS

1. To ascertain, in type 1 diabetic patients, whether glycemic variability measured as SDBG is an independent risk factor for the development of microvascular complications in addition to average glycemia, as assessed by HbA1c.

2. To study whether variability of HbA1c is an independent risk factor for the development of microvascular complications in addition to average glycemia assessed by HbA1c.

3. To compare glycemic control during basal insulin substitution with glargine versus CSII through CGMS monitoring.

4. To study the prevalence of severe hypoglycemia in relation to its possible risk factors in type 1 diabetic patients over a period of 14 years.

4 MATERIAL METHODS

4.1 BACKGROUND POPULATION

In 1984, the Department of Medicine at Danderyd Hospital had a catchment area comprising approximately 200,000 persons over the age of 15. In 1984, all patients attending the out-patient clinic were registered and the total came to 760 of which 434 were classified, based on clinical criteria, as type 1 diabetic patients. In 1999, 14 years later, the catchment area had expanded and the Department of Medicine was serving approximately 300,000 inhabitants, above the age of 15. All patients registered at the out-patient clinic and classified as type 1 diabetic patients totaled 847 (n=847). In 2005, the catchment area increased from about 400,000 to 560,000 persons above the age of 15. Patients registered at the out-patient clinic and classified as type 1 had increased to 1,118 in 2006.

Since 1996, the out-patient clinic for diabetes at Danderyd Hospital has used a locally developed quality record for clinical evaluation and for exporting data to the NDR. The NDR is a nationwide register for patients with diabetes. Annual reports in the field of diabetes mellitus describe the clinical activity focusing on the type of diabetes, treatment principles, blood glucose control, measured as HbA1c, and other clinically important factors such as blood pressure and blood lipids. The data shown below are one example from the local quality record.

The proportion of patients with type 1 diabetes (n = number of) who are treated with an insulin pump or the proportion treated with insulin analogs in four different years.

	1996 (n=738)	**2000** (n=960)	**2003** (n=1031)	**2006** (n=1118)
CSII	12.5%	12.2%	15.0%	16.7%
Short-acting analog	1.6%	50.9%	72.9%	93.1%
Long-acting analog	*N.A*	*N.A*	20.3%	72.8%

4.2 METHODS

4.2.1 Patients and Methods in Paper 1

From 442 consecutive type 1 diabetic patients who attended the diabetes out-patient clinic at Danderyd Hospital in 1990, 142 were randomly selected by date of birth and invited to participate in a study to measure blood glucose variability as determined by frequent capillary blood glucose values obtained through stratified home monitoring. One hundred of these patients agreed to participate in the study and thus formed a cohort in which SDBG was calculated, based on 70 measurements over a period of four weeks. The capillary tests were performed before breakfast, before lunch, before dinner, 1.5 h after dinner and before going to bed every second day for four weeks. The results of this investigation were reported elsewhere in 1994 (Moberg, Lins et al. 1994).
One hundred type 1 diabetic patients were told to measure their capillary blood glucose using the Reflolux® II M (Memory) meter before breakfast, before lunch, before dinner, 1.5 h after dinner and before going to bed every two days for four weeks, i.e. a total of 70 measurements. The glucose values, the corresponding insulin doses and the times of injections, together with significant events of daily life, i.e. hypoglycemia, illness and physical exercise, were entered into a diary. On the basis of all the available glucose values recorded during the four-week period, the standard deviation of blood glucose (SDBG) was calculated. The reproducibility of the SDBG was checked by repeating the same SMBG protocol in 20 of the patients after one year.

This group of 100 patients constituted the present study cohort, now re-analyzed after 11 years with respect to established risk factors for the onset and progression of micro- and macro-angiopathy, as well as peripheral neuropathy. During the follow-up period, the patients made visits two to four times a year to our out-patient clinic, according to our established clinical protocol.

Diabetic complications were defined and categorized in 1990 as follows. Nephropathy was defined as micro-albuminuria, MA (albumin excretion 30-300 mg/24 h) or albuminuria (albumin excretion exceeding 300 mg/24 h). Retinopathy was defined as proliferative diabetic retinopathy as determined by an expert in our research team using a blinded procedure. Peripheral neuropathy was defined as sensory neuropathy indicated by pathologic thresholds as revealed by neurometry and/or a vibration test with a tuning fork and mono-filament testing,
Autonomic neuropathy was defined as the clinical diagnosis of erectile disturbance, bladder dysfunction, orthostatic hypotension or gastroparesis. Unawareness of hypoglycemia was defined as a documented plasma glucose value of < 3.0 mmol/L without the ability to perceive symptoms of hypoglycemia. Macro-angiopathy was defined as blood pressure of > 140/85 and/or ongoing antihypertensive therapy, a clinical diagnosis of angina pectoris, intermittent claudication, myocardial infarction (MI) and/or cerebrovascular accident (CVA).

Clinical data, including medical history, diabetic complications, blood pressure, laboratory tests and pharmaceutical therapy, were extracted from each patient's medical file. HbA_{1c} was measured by liquid chromatographic assay (reference value for healthy subjects < 5.2%).

4.2.2 Patients and Methods in Paper 2

The same group of patients as in Paper 1 was included in this evaluation in which the onset and progression of micro- and macro-angiopathy, as well as peripheral neuropathy, were analyzed in relation to HbA1c and variability of HbA1c. Diabetic complications were defined and clinical

data were retrieved as in Paper 1. In all, 3,855 HbA1c values obtained over a period of 17 years were available for statistical evaluation.

4.2.3 Patients and Methods in Paper 3

A single-center, open, randomized, cross-over trial was conducted among type 1 diabetic patients on CSII for at least 6 months using a program for variable basal insulin infusion. The inclusion criteria were HbAlc of < 8.5% and an established routine to perform SMPG at least twice daily. The exclusion criteria were pregnancy, liver or kidney disease and progressive proliferative retinopathy. All the subjects were randomly assigned to receive either a morning dose of insulin glargine, comprising their average 24-h basal insulin requirement minus 2.4 units, which was delivered by the pump, or to continue as before for 4 weeks followed by a 1-week washout period and a cross-over, giving a total study period of 9 weeks. A two-period AB/BA cross-over design was used to evaluate the effect of glargine versus CSII on the mean glucose concentration, the glucose area under the curve and glucose variability. Each subject was randomly allocated to receive either basal insulin substitution with glargine or a pump in the first period. The two treatment periods were separated by a 1-week active washout. This active washout was used to avoid serious harm to the patients' health. The targets for fasting glucose and post-meal glucose were 4-6 and 6-10 mmol/L respectively. A small dose of insulin was also delivered by the pump during the glargine treatment to minimize the risk of catheter obstruction, as it was thought to be important that the patients retained their treatment routines to administer bolus insulin doses by the pump. Four different pumps were used by the patients: H Tron Plus (Roche Insulin Delivery Systems Inc., Fishers, IN) (n 1), MiniMed 507 (Medtronic MiniMed, Northridge, CA) (n 5), MiniMed 508 (Medtronic MiniMed) (n 8) and Deltec Cozmo (Smiths Medical International, Ashford, UK) (n 1). The patients were instructed to take their meal doses of direct-acting analog insulin (aspart or lispro) with the pump as previously. Throughout the study, the CGMS Gold (Medtronic MiniMed) provided profiles over a 24-h period on days 19-21, 26-28, 47-49 and 54-56. CGMS data were blinded until both treatment periods were finished. For each treatment phase, a statistical analysis of the glucose profiles was performed from the data collected from the CGMS, the mean glucose concentration, the glucose area under the curve and glucose variability. The glucose variability was calculated as the SD of plasma glucose (SDPG) and MAGE. HbAlc was determined by liquid chromatographic assay (reference value for healthy subjects < 5.2%) at the end of each treatment period.

4.2.4 Patients and Methods in Paper 4

At the beginning of 1985, all diabetic patients registered at the Department of Medicine, Danderyd Hospital, in 1984, were sent a questionnaire about hypoglycemic reactions during the previous year. The patients numbered 434 and they were all classified on the basis of clinical criteria as type 1 diabetic patients. The questionnaire was answered by 377 (87%) patients. The same procedure was applied in 1999. At this time, 847 patients were registered and classified as type 1 diabetic patients at our clinic. The second questionnaire was answered by 641 (76%) patients and 178 patients answered both questionnaires. The questionnaire related to events of SH, unawareness of hypoglycemia, nocturnal episodes, pharmacological treatment and SMBG. SH was defined as an episode for which help from another person was required. Unawareness of hypoglycemia was defined as a blood glucose value of < 3 mmol/ l without the ability to perceive symptoms of hypoglycemia. Data relating to medical history were collected from medical files. Over the study period, the patients routinely attended the out-patient clinic two to three times a year. Diabetic complications were defined as follows; retinopathy as reduced visual acuity (< 0.5 bilateral), nephropathy as albuminuria = 300 mg/24 h, sensory and autonomic neuropathy as a clinical diagnosis of neuropathy. Renal function was assessed by calculating the creatinine clearance according to the formula (140-age) weight (kg)/7 s-creatinine (mg /dl). Macro-angiopathy was defined as hypertension, blood pressure of > 140/85 mm/Hg and/or ongoing anti-hypertensive therapy, a clinical diagnosis of angina, claudication, myocardial

infarction (MI) and/or cerebrovascular disease. Data on the patients who participated in the first questionnaire study but not in the second were also collected and cause of death was obtained from the National Department of Statistics and/or the results of forensic autopsies. Biochemical analyses: HbA1c was determined by an isoelectric focusing method, reference value for healthy subjects < 8.7% in 1982-1984, by liquid chromatographic assays, reference value < 5.6% in 1984-1990 and < 5.2% from 1991. Due to the different methods of HbA1c analysis used in the course of the study, values were recalculated using the upper limit of reference.

4.2.5 Questionnaire used in Thesis

The questionnaire used in 1985 and 1999 consists of 28 questions relating to treatment, incidence of hypoglycemia and psychosocial factors. Some of these questions, are as follows.

How often did you experience severe hypoglycemia (see definition) during the last year?

1. Never
2. At least once but fewer than five times
3. 1-3 times/month
4. 1-3 times/week
5. 3 times/week

Have you been to the emergency room due to SH during the last year?

1. Yes
2. No

If yes, how many times?

…….times

Do you modify your insulin dosage depending on the level of the b-glucose?

1. Yes
2. No

If yes, how often?

1. Daily
2. Weekly
3. Monthly

How often do you measure your b-glucose?

1. Daily
2. 2-3 days/week
3. 2-3 days/month
4. Less often
5. Never

Have you measured a b-glucose of < 3 mmol/l without having any hypoglycemic symptoms?

1. Never
2. At least once but fewer than 5 times during the last year
3. 1-3 times/month
4. 1-3 times/week
5. > 3 times/week

Demographic and treatment data of the cohorts with type 1 diabetes mellitus (DM) in 1984 and 1998 and the study group

Number of patients	Cohort of 1984 (n = 377)	Cohort of 1998 (n = 641)	Study group in 1984 (n = 178)	Study group in 1998 (n = 178)
Gender (percent male)	56	51	54	54
Age (years ± SD)	37.8 ±13.1	44.2 ±13.7	35.0 ± 9.8	49.0 ±9.8***
Duration of DM (years ±SD)	18.6 ± 11.9	22.4 ±13.5	17.9 ±10.9	32.3 ±10.9***
HbA1c (% ± SD)	7.9 ± 1.5	7.4 ±1.4	7.6 ± 1.3	7.4 ± 1.1*
Severe hypo-glycemia (%)	20	21	17	27*
Insulin units/kg bodyweight/day	0.54 ± 0.14	0.60 ± 0.19	0.57 ± 0.17	0.56 ± 0.14
Multiple injection therapy (%)	67	98	71	98***
SMBG daily (%)	13	49	17	48***
Nocturnal events (%)	71	80	76	83*
Reduced awareness (%)	38	46	40	54**

Statistical calculation between the cohorts in the study group: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
Including pump treatment
SMBG, self-monitoring of blood glucose.

5 STATISTICAL ANALYSES

5.1 PAPER 1

Statistical significance was calculated using Student's two-tailed *t* test for paired observations for continuous data and the chi-square test for association for categorical data. Values of SDBG were applied in statistical analyses (logistic regression, Cox regression) prior to and after adjustment for mean blood glucose (measured as HbA1c), age and duration of diabetes. The prevalence of complications at the start of follow-up was analyzed by logistic regression. The complication-free cohort was analyzed with respect to the development of complications by Cox regression, in which patients were censored when lost to follow-up or deceased.

5.2 PAPER 2

The standard deviation of HbA1c (variability) and the mean HbA1c (overall level) was calculated, for each patient, using their measurements over time. To evaluate the effect of variability and the overall level of HbA1c on the total number of complications, a generalized linear model was fitted. We assumed that a Poisson distribution with a log link as the outcome is a positively skewed discrete continuous variable. The total number of complications consisted of proliferative diabetic retinopathy, albuminuria, micro-albuminuria, peripheral neuropathy, gastroparesis and erectile dysfunction, with each variable categorized into not-present and present. Patients who died during follow-up were not included in the analysis, as they have missing information regarding the number of complications. As covariates, age at baseline, disease duration and mean BMI were included in the model. The results are presented as risk ratios (RR) with 95% confidence intervals and p-values. The RRs were transformed to clinically relevant units. A t-test for independent groups, assuming unequal variances, was used to compare the patients who were alive with those who died during follow-up in terms of mean variability and mean overall HbA1c. IBM SPSS statistics (version 18.0, SPSS Inc., Chicago) were used for analyses and graphs.

5.3 PAPER 3

ANOVA for repeated measures was performed to estimate the difference between patients treated with glargine and CSII in the outcomes for AUC, SMPG, CGMS, number of episodes and variability. The outcomes were analyzed in separate models. A period by treatment interaction was included in all models to maximize the efficiency of the estimation of the treatment difference. An active washout was chosen because an absence of treatment would be harmful for the patients. The length of the washout period was clinically long enough to avoid any carry-over effects. Normal probability plots and residual plots were used to investigate the model assumptions underlying an ANOVA. P-values of less than 0.05 were regarded as statistically significant. All analyses and graphs were produced using the Statistica statistical software (version 7.0, StatSoft, Inc., Tulsa, Oklahoma).

5.4 PAPER 4

Statistical significance was evaluated by means of Student's two-tailed *t*-test for paired observations when normally distributed and the chi-square test for association when not. To determine the significance of risk factors of SH, a stepwise logistic multiple regression analysis was performed using severe hypoglycemia as the dependent variable. P–values of less than 0.05 were considered significant. Unless otherwise stated, the data are given as means ± SD.

6 RESULTS AND DISCUSSION

6.1 IS VARIABILITY OF BLOOD GLUCOSE IMPORTANT?

In a subgroup analysis of the DCCT cohort, it was demonstrated that 8% of intensively treated subjects as compared to 20% of non-intensively treated patients with similarly elevated HbA_{1c} levels developed retinopathy within nine years, a finding that has been quoted in support of the notion that there is "something unique" about intensive treatment independent of HbA_{1c} levels (Bloomgarden 2002). The question of why this difference exists then arises; it is possible to speculate that the difference may be due to reduced glycemic variability in the intensively treated group. Clinical studies have documented that long-term variability of fasting glucose is an independent predictor of mortality in patients with type 2 diabetes (Muggeo, Verlato et al. 1995) and data extracted from the DCCT cohort suggested that, while updated mean blood glucose was the primary risk factor for mortality, the mean amplitude of glycemic excursions recorded at baseline in one multivariate analysis also made a significant contribution to mortality (Service and O'Brien 2001). Additional support for the idea that glucose variability affects the risk of microvascular complications comes from another study where the incidence of retinopathy in a group of adolescents with type 1 diabetes appeared to fall substantially between 1990 and 2002, despite little change in HbA1c levels during the study period; the authors concluded that the move to multiple injection regimens over time may have contributed to this improvement by reducing glycemic fluctuations despite stability in the mean glucose concentration (Mohsin, Craig et al. 2005).
However, Kilpatrick and coworkers (Kilpatrick, Rigby et al. 2006; Siegelaar, Kilpatrick et al. 2009) have published results from a statistical analysis of the large DCCT database, reporting that HbA1c, but not glucose variability, was associated with a long-term risk of developing micro-angiopathy. The authors concluded that pre- and postprandial glucose values were equally predictive of small-vessel complications of type 1 diabetes.

In our analysis of a group of type 1 diabetic patients followed for 11 years, we found that SDBG was an independent predictor of the prevalence of peripheral neuropathy, as well as a predictor of the incidence of peripheral neuropathy of borderline significance. SDBG was also a predictor of the incidence of hypoglycemic unawareness. We failed to establish a significant relationship between SDBG and retinopathy or nephropathy. Since we defined retinopathy very narrowly as proliferative retinopathy and the incidence of this, as well as the incidence of nephropathy, appeared to be quite low compared with data from some other clinic-based studies, we can speculate that our study was insufficiently powered to detect such relationships – if they exist. Other study limitations that could change the interpretation would, for example, be the small number of patients, but the follow-up period is considerably long, 11 years. There are also confounding factors such as hemorheological abnormalities (platelet activation, fibrinogen levels) and endothelial cell dysfunction (von Willebrand factor, cell adhesion molecules) that we have not measured in this study. This was a clinical observational study and at our clinic we do not measure these factors routinely. The focus of this study was to see whether we could find any new information regarding the development of microvascular complications by using blood glucose variability measured as SDBG. We did not find any correlation between smoking and microvascular complications. The question about possible reversed causality is difficult to answer, but our interpretation of the result is that variability measured as SDBG has an impact on peripheral neuropathy rather than the opposite. Kilpatrick and co-workers (Kilpatrick, Rigby et al. 2006; Siegelaar, Kilpatrick et al. 2009) did not find any correlation between glucose variability and the relative risk of developing micro-angiopathic (nephro- and retinopathy) complications. They used 7-point blood-glucose profiles taken at 3-month intervals yielding an aggregate of 24,652 capillary blood glucose profile values between pre-breakfast (0700) and

bedtime (2200) hours. Instability of blood glucose (within-day SD) was calculated as the SD of daily blood glucose, variability over time was estimated as the SD of the mean blood glucose measurements measured at each quarter. They found that glucose variability did not play a role in the development of micro-angiopathic complications and concluded that only elevation of mean glucose over time as expressed by HbA1c was associated with a proportionally greater risk of developing micro-angiopathy in the long term. In a previous clinical study, a relationship between blood glucose excursions and painful neuropathy was documented (Oyibo, Prasad et al. 2002). At a given level of HbA1c, high variability in measured glucose will increase the number of both hyper- and hypoglycemic excursions. The latter, if recurrent, may induce a state of hypoglycemic unawareness which in itself is a major risk factor for severe hypoglycemic events in patients with type 1 diabetes (Oyibo, Prasad et al. 2002; Bragd, Adamson et al. 2003; Cryer, Davis et al. 2003).

In the present study, we were able to verify that SDBG was a highly significant predictor of the incidence of hypoglycemic unawareness. This raises the question of a putative relationship between hypoglycemia and peripheral neuropathy. In animal experiments, hypoglycemia may cause a distal axonopathy including both degenerative and regenerative events; in this respect, motor axons appear to be more vulnerable than sensory axons (Mohseni 2001). In our previous review of the literature, we found no studies showing that the development of peripheral neuropathy in diabetic humans could be unequivocally attributed to hypoglycemia (Lins and Adamson 1993). Other mechanisms for the development of peripheral neuropathy could be the activation of the polyol/sorbitol pathway or the activation of oxidative stress. They are, however, related to the level of the HbA1c.

6.2 IS LONG-TERM LIABILITY OF GLYCEMIC CONTROL, AS DETERMINED BY THE VARIABILITY OF HBA1C, RELATED TO DIABETIC COMPLICATIONS?

There is still a debate about whether short- or long-term glucose variability constitutes an additional risk of microvascular complications.

In a study performed by our research team on a cohort of 100 type 1 diabetes patients, we found that glucose variability measured as SDBG was related to the long-term risk of developing peripheral neuropathy, ($P = 0.03$) hazard ratio 2.34 (1.06-5.20), as well as being a predictor of the incidence of peripheral neuropathy at borderline significance, ($P = 0.07$) hazard ratio 1.73 (0.94-3.19) (Bragd, Adamson et al. 2008). On the contrary, Kilpatrick and coworkers (Kilpatrick, Rigby et al. 2006) have published several statistical analyses of the large DCCT database, reporting that glucose variability was not associated with the risk of developing micro-angiopathy.(Kilpatrick, Rigby et al. 2006; Siegelaar, Kilpatrick et al. 2009).
However, in another analysis of the DCCT database, they were able to show that variability in HbA1c was correlated to an increase in microvascular complications (Kilpatrick, Rigby et al. 2008).

In 2009, a Finnish study group presented data on the variability of HbA1c. They performed an observational multicenter study of 2,107 patients and found in a Cox regression model that the SD of HbA1c was independently associated with the progression of renal disease and of CVD events among patients with type 1 diabetes mellitus (Waden, Forsblom et al. 2009). Their study was an observational study and would probably reflect the normal clinical setting better in terms of HbA1c variability compared with an interventional setting like that in the DCCT study.
In the Finnish study, the endpoints were nephropathy and CVD and no other microvascular complications. For this reason, we have now analyzed our observational study cohort regarding variability in HbA1c and the development of all microvascular complications. During the

follow-up period, HbA1c was measured as a normal clinical routine at our out-patient clinic; 3,855 HbA1c tests were collected, giving a mean of 2.3 values per year and patient.
We hypothesize in the present study that HbA1c variability measured as SD is related to the development of microvascular complications in subjects with type 1 diabetes.

In this study, we found that long-term glucose variability measured as the SD of HbA1c was related to an increased number of microvascular complications. Our finding supports the study presented by Waden et al.(Waden, Forsblom et al. 2009), where, in a Cox regression model, they showed that the SD of HbA1c was independently associated with the progression of renal disease and of CVD events among patients with type 1 diabetes mellitus. Compared with our study, they analyzed nephropathy among the microvascular complications. In our study, microvascular events of all kinds were included and were of importance for the statistical association. Our study consisted of only 100 patients at the start, although we had a considerably long follow-up period of 11 years. When the results were analyzed, a clear statistical significance was revealed and we therefore believe it is of clinical relevance.
However, to answer the question of causality, an interventional study, such as the DCCT with HbA1c variability as the end-point, is needed.
Our finding is also consistent with the study by Kilpatrick et al. who reported that variability in HbA1c adds to the mean glucose value in predicting microvascular complications in type 1 diabetes (Kilpatrick, Rigby et al. 2008). Their study consists of a huge number of HbA1c values which makes the statistical analysis very strong and the result is therefore of clinical relevance. Moreover, in a recent report from the Oxford Regional Prospective Study and the Nephropathy Family Study, comprising a total of 1,232 participants, it was concluded that HbA1c variability was an independent variable that added to the effect of HbA1c on the risk of micro-albuminuria in young people with type 1 diabetes (Marcovecchio, Dalton et al. 2011).

The question of whether short-term or long-term variability might add to long-term glycemia as a risk of diabetic complications has been the subject of debate for many year. In our group of patients studied for a period of eleven years, it appears that both long-term glucose variability and short-term glucose variability have an impact on the development of microvascular complications. This was shown to be independent of mean blood glucose measured as HbA1c and there was no correlation between HbA1c variability and SDBG. Our patients already had long disease duration of approximately 20 years when they were included, which differs from the DCCT study, making the patients in our study more prone to develop a larger number of complications during the follow-up period. The patients were followed up for a long period, which we believe should compensate for the relatively small number of patients in our study. The study by Wadén et al. also revealed a correlation between HbA1c variability and macrovascular complications (CVD), which we did not find. However, the number of macrovascular events in our study was small and a correlation would therefore have been difficult to find.

The mechanisms for the development of diabetic complications are usually described as arising from sustained periods of hyperglycemia which lead to the intracellular overproduction of superoxide. The formation of superoxide is the key event in the activation of all the other pathways, such as the polyol/sorbitol pathway flux, increased advanced glycated end (AGE) product formation, increased hexosamine flux, the activation of oxidative stress and so on (Brownlee 2001). The effect of variable blood glucose, with periods of high glucose levels followed by periods of low levels, or vice versa, might be more deleterious in this respect than continuous hyperglycemia. There are studies that have shown that glucose variability in vitro triggers oxidative stress more than sustained periods of hyperglycemia (Piconi, Quagliaro et al. 2006). Another possible mechanism might be the theory of “metabolic memory”. Periods of

higher glucose levels could induce harmful effects later on, even though the glucose level at that time has been normalized, “metabolic memory” (Ihnat, Thorpe et al. 2007).

6.3 DOES BASAL INSULIN SUBSTITUTION THERAPY WITH CSII GENERATE GLUCOSE PROFILES THAT ARE “SUPERIOR” TO THOSE ACHIEVED WITH INSULIN GLARGINE?

Since the introduction of CSII, this type of treatment has been regarded as the “golden standard” for achieving near normoglycemia without increased episodes of hypoglycemia. Several studies have shown that CSII is superior to multiple insulin injection therapy (MDI) treatment with human insulin in terms of glucose control and also in reducing episodes of hypoglycemia (Bode, Steed et al. 1996; Linkeschova, Raoul et al. 2002; Hoogma, Hammond et al. 2006). However, the development of insulin analogs, e.g. glargine (Lantus, Sanofi-Aventis Pharmaceuticals Inc.), has improved MDI treatment compared with MDI with human insulin. MDI treatment with glargine produces improved glycemic control and fewer episodes of hypoglycemia compared with MDI with human insulin (Ratner, Hirsch et al. 2000). The question of whether MDI treatment with glargine is comparable to CSII treatment therefore arises. When comparing MDI on glargine with CSII, it has been shown that CSII improves glucose control among patients with type 1 diabetes and also reduces the risk of hypoglycemia (Hirsch, Bode et al. 2005).

The recent development of a CGMS represents an improvement in the process of evaluating the glucose profiles of patients with type 1 diabetes. The greatest advantage with CGMS compared with the self-monitoring of plasma glucose (SMPG) is the opportunity also to monitor the night when the patient is asleep. It is generally held that basal insulin substitution with CSII produces less variable glucose levels than with long-acting insulin analogs, e.g. glargine, in patients with type 1 diabetes, although this has hitherto not been convincingly demonstrated in adults by continuous glucose monitoring (Bruttomesso, Crazzolara et al. 2008). However, in a prospective study performed on young children with type 1 diabetes, CSII reduced glucose variability measured as MAGE compared with MDI treatment with glargine and lispro (Alemzadeh, Palma-Sisto et al. 2007). The aim of our study was to compare the glucose control as determined by the CGMS in type 1 diabetes patients on CSII with or without the supplementary basal insulin analog, glargine. We conducted an open, randomized, cross-over trial with 15 type 1 diabetics using CSII.

In this study, the most prominent finding was the improvement in the glucose profile among the patients when using CSII. During CSII treatment, the patients were closer to normoglycemia compared with glargine treatment. There were a few more hypoglycemic (<3.5 mmol/l) episodes when using CSII, but there was no significant difference with glargine regarding the time spent in the hypoglycemic range (<3.5mmol/l). The small dose of insulin delivered by the pump during the glargine treatment was designed to prevent pump malfunction and to enable the patients to retain their normal insulin treatment routines and thereby minimize the changes between the two treatment arms. The patients were also instructed to take their meal doses, of a direct-acting analog insulin aspart or lispro, with the pump as previously. When calculating variability, SDBG was used because it is an easily available glucose index for glucose variability and the CGMS program also includes calculations of SD. In our study group, we have previously used SD to assess glucose variability (Moberg, Kollind et al. 1993; Moberg, Lins et al. 1994). MAGE is, however, the “golden standard“ for glucose variability and we therefore also used this method (Service, Molnar et al. 1970). In our study, there was no difference in variability between glargine and CSII. This finding could perhaps be due to the fact that the study was underpowered; only 15 patients completed the study. However, when analyzing the

results, it is not likely that a few more patients would change the outcome to any significant degree.
One advantage of this study was the use of CGMS when monitoring the patients. This meant that we were also able to include the nighttime values in the calculation of variability. It is important to know that the "golden standard" for the calculation of glucose variability, MAGE, was first performed on patients with continuous glucose monitoring by Service in 1970. When performing only SMBG, there is a risk of underestimating or overestimating glucose variability due to the fact that the nighttime values cannot be seen. The night is a "blind spot".

6.4 WHAT HAS THE USE OF MULTIPLE INJECTION THERAPY AND SMBG MEANT FOR THE PREVALENCE OF SEVERE HYPOGLYCEMIA IN CLINICAL PRACTICE?

Over the last 20 years, new therapeutic strategies have been introduced in the management of type 1 diabetic patients; they include the use of multiple-injection therapy, new insulin analogs and self-monitoring of blood glucose (SMBG), as well as the intensified education of patients and relatives. All these factors may help to improve the glycemic control and the quality of life at a low risk of hypoglycemia.

In the DCCT study, a strong inverse relationship between the HbA1c level and the incidence of SH in the intensively treated patients was documented and the number of prior episodes of hypoglycemia was the strongest predictor of the risk of future episodes. Furthermore, long diabetes duration, low stimulated C-peptide levels and a high insulin dose were associated with SH. In the DCCT, half of all SH episodes occurred during sleep and one third of daytime episodes occurred without apparent warning (Clarke, Cox et al. 1995). Although a number of risk factors for SH were identified in the DCCT, together they explained less than 10% of the variance (Bott, Bott et al. 1997).
In clinical practice, other variables to consider with respect to SH include psychosocial factors, as it has been suggested that these factors play an important role in the successful self-management of diabetes (Heller 2000).
When comparing the fairly diverse prevalence and incidence figures of SH presented in different studies (Tattersall 1999), the HbA1c profile of each study population is one factor that has to be taken into consideration.
The results from our study group showed an increase in the prevalence of SH from 17 to 27 percent during the 14 years of observation. An inverse correlation to the HbA1c level and a correlation to unawareness were also found in our group. Our figures for unawareness are derived from self-reporting and it is important to remember the risk of bias when using such data. Clarke and colleagues addressed this issue in a prospective evaluation of the frequency and severity of hypoglycemic episodes in type 1 diabetic subjects who declared themselves to have reduced awareness of hypoglycemia and concluded that these patients are generally correct (Clarke, Cox et al. 1995). It has even been suggested that estimates of the prevalence of unawareness based on patient questionnaires may underestimate its extent (Heller 2000). Moreover, our data are very much in line with those of other investigators (Pramming, Thorsteinsson et al. 1991; Frier 1999). In a previous study, hypoglycemia unawareness has been shown to predispose to a sixfold higher rate of SH as compared to that of patients with normal awareness (Gold, MacLeod et al. 1994). Reduced hypoglycemia awareness was reported by as many as 54% in our study group, with a mean duration of diabetes of 32 years. This corresponds to an annual increase in the frequency of unawareness of 1% between our cross-sectional surveys.

SMBG became a “standard” procedure in the management of type 1 diabetic patients while this study was ongoing. There are two main reasons for this; one is the need frequently to adjust insulin doses in intensive therapy and the other is the need to detect low blood glucose since the occurrence of SH is strongly related to the frequency of low blood glucose readings (Cox, Kovatchev et al. 1994; Kovatchev, Cox et al. 2000). Allen and co-workers reported that SMBG independently predicted frequent episodes of hypoglycemia but not SH (Allen, LeCaire et al. 2001). This is consistent with our present findings that daily SMBG was not related to SH, in spite of the fact that as many as 48% of our patients performed SMBG on a daily basis.
Notably, in our statistical evaluation, neither age nor the duration of diabetes was significantly related to SH in our study group, while such relationships were found in the cohorts of 1984 and 1998 respectively.
It has been demonstrated that nephropathy (Muhlhauser, Toth et al. 1991; Bell and Cutter 1994) and neuropathy (Bell and Cutter 1994; Stephenson, Kempler et al. 1996) are related to SH. The prevalence of overt nephropathy and renal failure was low in our study population and we were thus unable to further elucidate the role of nephropathy in this respect.

7 CONCLUSIONS AND FUTURE PERSPECTIVES

7.1 CONCLUSIONS PAPER 1

We conclude that the variability of blood glucose may be of importance for the development of peripheral neuropathy in subjects with type 1 diabetes and that nerve tissue might thus be particularly vulnerable to glycemic variability. However, the issue of the correlation between blood glucose variability and the development of microvascular complications still remains open.

7.2 CONCLUSIONS PAPER 2

We conclude that the variability of HbA1c may be of importance for the development of microvascular complications in subjects with type 1 diabetes.

7.3 CONCLUSIONS PAPER 3

It is concluded that CSII provides superior glucose control as compared to glargine, with a lower mean blood glucose ($p=0.002$), longer periods of glucose values within target ($p=0.034$) and a lower HbA1c ($p=0.018$) on a somewhat smaller insulin dose (n.s.). There was, however, no significant difference with respect to glucose variability calculated as SDPG or MAGE.

7.4 CONCLUSIONS PAPER 4

We conclude that, in spite of the more frequent use of multiple injection therapy and more frequent SMBG, the prevalence of SH increased by more than 50% over 14 years. A multiple logistic regression analysis of risk factors for SH explained less than 10 percent of the variance, giving significance only to unawareness of hypoglycemia and HbA1c.

7.5 FUTURE PERSPECTIVES

How can we increase the translation of research results into clinical practice and improve the medical care of patients?

- Further studies regarding short-term and long-term glucose variability would be welcomed and are important.
- In clinical practice when treating patients with type 1 diabetes, I recommend that a value of the patient's glucose variability should be recorded alongside HbA1c.
- The recording of glucose variability should be performed with a "blinded" CGM.
- I recommend SD as a measure of glucose variability due to its simplicity and the fact that all CGM software contains SD in the program.
- In our study group, we are going to perform a randomized, double-blind, cross-over study evaluating the difference in glucose variability between placebo and domperidone with CGMS.
- Perhaps we should promote treatment recommendations for glucose variability, as we have with HbA1c and blood pressure measurements.
- It is important that, even in the future, The Dental and Pharmaceutical Benefits Agency (TLV) have some kind of reimbursement for glucose sensors and CSII.
- It is to be hoped there will be some kind of "closed loop" system on the market in the near future. (5-10 years?)

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