Alternative Assessment of **Glycemic Control**

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Alternative Assessment of Glycemic Control

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(met een samenvatting in het Nederlands)

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Content

Chapter 1

General Introduction

Diabetes mellitus is a chronic disease which is associated with development of microvascular (retinopathy, nephropathy or neuropathy) and macro-vascular (cerebrovascular, coronary or peripheral artery disease) complications. Optimal glycemic control is the cornerstone of diabetes management for prevention of diabetes complications and therefore glucose monitoring is needed. While in the past glucose measurements were restricted to semiquantitative urinary glucose measurements; self-monitoring of blood glucose and subsequently HbA1c became available 3 decades ago. These parameters have substantially improved quantification of glycemic control[1]. And HbA1c has remained the main treatment target used to quantify glycemic control in diabetes mellitus since.

HbA1c indicates the percentage of glycated hemoglobin and is a reflection of the mean glucose level in a patient[2]. For instance an HbA1c of 53 mmol/mol (7%) corresponds with a mean glucose level of 9.5 mmol/L[2]. Large clinical trials investigating the effect of improving glycemic control like the U. K. Prospective Diabetes Study (UKPDS) and the Diabetes Control and Complications Trial (DCCT) showed that the risk for complications diminishes when HbA1c decreases[3, 4].

However, the exact level of HbA1c as treatment target has been subject to intense debate. Although lowering of HbA1c results in less complications, it is not simply the lower the better. This was shown in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial in which a higher mortality was present in the intensive-therapy group (aiming to reach an HbA1c < 6.0% [42mmol/mol] in a very short period) compared to the standard-therapy group (aiming at HbA1c 7.0 – 7.9% [53-63 mmol/mol])[5]. Furthermore, a retrospective study in type 2 diabetes patients has shown a U-shaped curve for HbA1c and mortality, with an optimum HbA1c level of 7.5% (58 mmol/mol)[6]. This suggests that glycemic control is much more complex than HbA1c alone and that just reducing HbA1c, without taking into account other parameters of glycemic control such as glycemic variability does not necessarily improve outcome.

The measurement of HbA1c has some disadvantages. HbA1c can be less reliable in certain clinical conditions such as anemia, renal insufficiency or hemoglobinopathies[1]. It only reflects glycemic control in the past few months and does not provide information about hyperglycemia for a considerably longer period of time[1]. Since it is an index of the mean glucose, it does not provide information about actual glucose levels, glucose peaks, glucose nadirs and fluctuation of glucose over time.

Therefore glycemic control is too complex to be captured in a single value (HbA1c or mean glucose).

This thesis aims to investigate usefulness of alternative parameters of glycemic control, such as glycemic variability (showing actual glucose levels) and advanced glycation end products (another more long-term biomarker of mean glucose) in treatment of patients with diabetes.

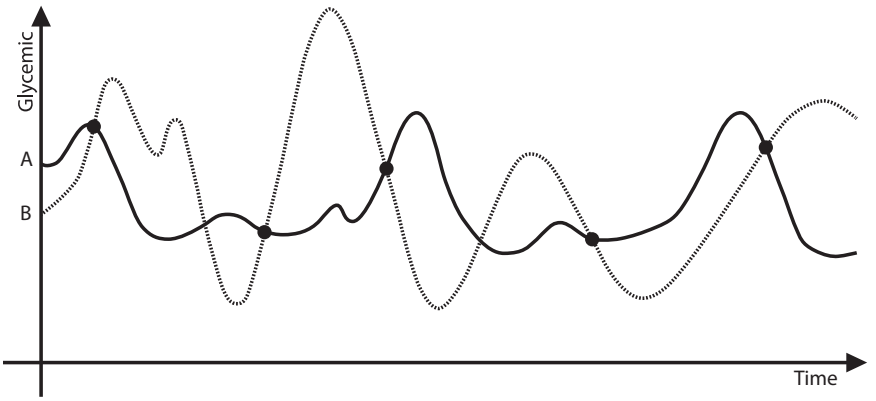
Continuous glucose monitoring and glycemic variability

In addition to increased glucose levels, diabetes is associated with fluctuation of blood glucose (glycemic variability). In subjects without diabetes, glucose levels vary only about 50% throughout the normal day, while in diabetes patients this may be 10-fold leading to higher glycemic variability[1].
Glycemic variability can be measured by a continuous glucose monitoring system, which comprises a needle (containing a glucose-dependent enzyme generating glucose-dependent electrical currents) which has to be inserted into subcutaneous fat. A transmitter is connected to the needle and a separate receiver stores (off-line continuous glucose monitoring) or displays (real-time continuous glucose monitoring) the glucose profile.

Example of real-time continuous glucose monitoring; the needle in the subcutaneous space is to the left and the receiver (which can be part of the continuous insulin infusion pump) is shown at the belt of the patient with real-time display of glucose values

While self-monitoring of blood glucose gives only a snapshot of the glucose levels during the day, continuous glucose monitoring shows glucose levels in a continuous fashion (usually every 5 minutes), showing hyperglycemia and hypoglycemia that would otherwise have been missed. Off-line continuous glucose monitoring (in which glucose values are blinded to the patient) can be used to discuss the glucose profile and variations with the patient afterwards

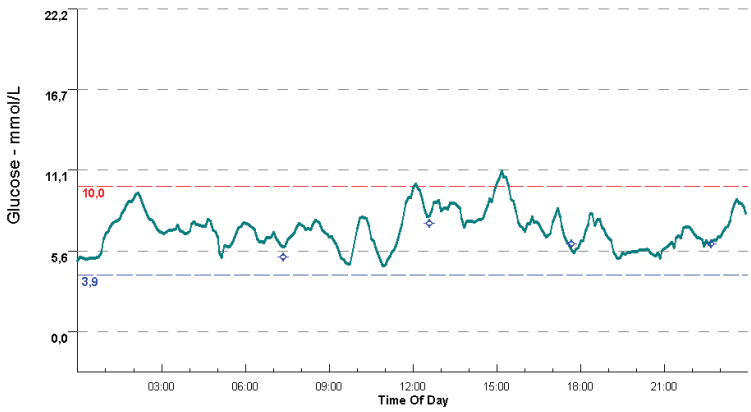
and is basically used as an educational tool. Real-time continuous glucose monitoring can be used by a patient to make direct treatment decisions from the glucose values that can be observed every 5 minutes. Real-time continuous glucose monitoring is able to not only show actual glucose levels, but also to show the trend of the slope (are glucose levels increasing or decreasing). Direct treatment decisions based on the real-time observed hypoglycemia or hyperglycemia have the potential to directly improve glycemic control (HbA1c) as well as glycemic variability. We reviewed the potential of this device to improve these parameters in diabetes patients in chapter 4.



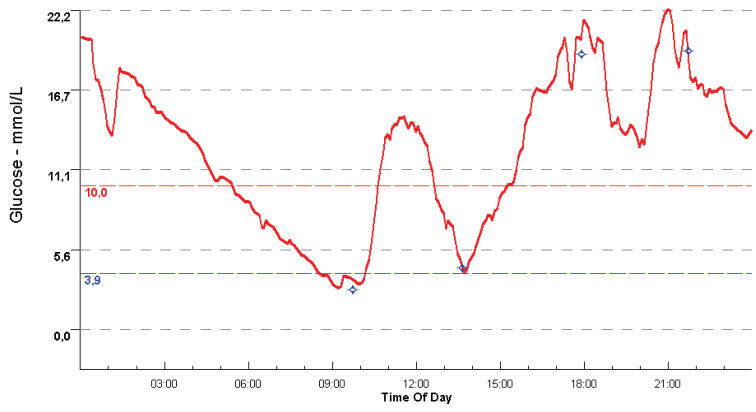
Snapshot nature of self-monitoring of blood glucose versus continuous glucose monitoring.
Black dots show self-monitoring of blood glucose measurements. As can be derived from this figure, many hypo- and hyperglycemia's are missed by just self-monitoring of blood glucose measurements.

With continuous glucose monitoring we are not only able to visualize glycemic variability but also to quantify this. However, the multitude of proposed parameters and the lack of a gold standard shows the difficulty of clearly quantifying glycemic variability. Commonly used parameters are standard deviation (SD) or coefficient of variation (CV) of blood glucoses, mean amplitude of glycemic excursions (MAGE, a measure of the variation around the mean blood glucose greater than 1 SD), continuous overall net glycemic action ($CONGA_{n,}$ SD of summated differences between glucose levels during a certain time period and n hours apart) and mean of daily differences (MODD, mean absolute value of differences between glucose values between two separate days)[7].

In chapter 2 we investigated different parameters of glycemic variability and the association between these parameters in our cohort to suggest a preferred parameter. We also investigated differences in glycemic variability between type 1 and type 2 diabetes. Furthermore, we used this chapter to investigate our hypothesis that glycemic variability would increase with progression of the disease in type 2 diabetes. While we hypothesized that in type 1 diabetes it would remain stable, since in existing type 1 diabetes beta cell mass is usually already decreased to a minimum in a short period at the beginning of the disease.



Example of a patient with type 2 diabetes treated with diet showing low glycemic variability



Example of a patient with type 1 diabetes on intensive insulin with high glycemic variability

Glycemic variability has been suggested to be an important risk factor for diabetes complications[8]. Although its relevance is controversial since studies investigating the association between glycemic variability and diabetes organ complications show inconsistent results[9-15].
Especially when chronic diabetes complications and hypoglycemia are present, patients with diabetes have a decreased quality of life as compared to the general population[16-19]. From clinical observations it seems that patients also suffer from large glycemic variability because this adds to non-predictability of the disease, decreased perceived ability to control it and non-satisfaction with treatment and therefore reduced overall quality of life. In chapter 3 we investigated the association of glycemic variability with quality of life in our large cohort of type 2 diabetes patients. If higher glycemic variability is associated with worse quality of life, this would emphasize it as an important clinical problem and reducing glycemic variability could be a worthwhile target for treatment and potential endpoint in intervention trials.

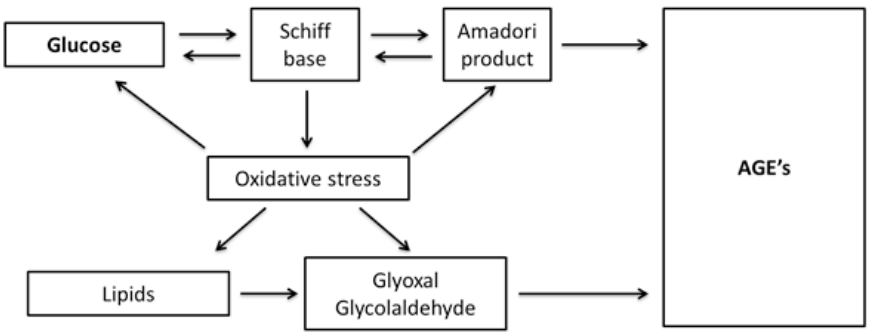
Potential interventions to improve glycemic variability; DPP4-inhibitors

Another strategy to reduce glycemic variability apart from real-time monitoring could be adding a DPP4-inihibitor to insulin treatment. DPP4-inhibitors act via the Glucagon-like peptide-1 (GLP-1)-pathway. After the ingestion of food, GLP-1 is normally secreted from the L-cells in the small intestines. GLP-1 stimulates the beta cells in a glucose-dependent fashion. Similarly it decreases glucagon production of the pancreatic alpha cells, also glucose dependent, thereby inhibiting paradoxal post-prandial hyperglucagonemia present in diabetes patients. Normally GLP-1 is rapidly degraded by the enzyme dipeptidylpeptidase IV (DPP-4). Oral inhibitors of DPP-4 such as vildagliptin inhibit this enzyme, thereby increasing the levels of GLP-1. This could theoretically be important to improve glycemic variability, since glycemic variability is determined by hyperglycemic and hypoglycemic excursions. If beta cells are stimulated in a glucose dependent fashion, this could reduce hyperglycemic excursions. This effect can be augmented by the influence of GLP-1 on alpha cells (less paradoxal post-prandial glucagon production) which adds to the effect of less hyperglycemia. There is also evidence that alpha cells react more appropriately to low glucose levels when DPP4-inhibitors are present, which can lead to less or less severe hypoglycemic excursions[20]. A decrease in glycemic variability in type 2 diabetes patients has indeed been reported before in single-arm trials investigating the effect of addition of a DPP4-inhibitor to insulin[21, 22].

Multiple randomized-controlled trials investigating the effect of DPP4-inhibitors added to *existing* insulin on HbA1c have been performed [23-32], however no data exist about vildagliptin use at the *start* of insulin treatment in patients with type 2 diabetes and no data exist from randomized trials about reduction of glycemic variability. Adding a DPP4-inhibitor at the start of insulin treatment could especially be interesting, because it is in this period that considerable residual beta cell function is still present. The benefit of such a strategy could not only be less glycemic variability, but also a lower required insulin dose for glycemic control. In chapter 5 we describe our randomized-controlled trial to investigate this.

Advanced Glycation End products

Whereas HbA1c is a reflection of the glucose levels of the past months, advanced glycation end products (AGEs) are a more long-term reflection (many years) of hyperglycemia. AGEs are modified long-lived tissue proteins that accumulate in body tissue during aging. These glyco-oxidation products are formed by different pathways. One is the Maillard reaction, in which glycated proteins are formed by a series of sequential reactions between glucose and proteins. Another pathway is formation by reactive carbonyl compounds (oxidative stress).



Schematic representation of the formation of some common advanced glycation end products (AGEs).

Accelerated accumulation of AGEs is seen with glycemic or oxidative stress and decreased renal clearance, resulting in AGE accumulation in patients with diabetes mellitus, patients admitted to the intensive care unit or patients with renal failure[33-35]. In patients with diabetes, AGEs in skin biopsies predict the

progression of microvascular complications (retinopathy and nephropathy), and the level of serum AGEs predicts mortality rate (all-cause and cardiovascular)[36, 37]. However these studies use invasive methods to measure AGEs, whereas AGE-accumulation can easily be assessed non-invasively by the AGE-reader[38] using auto-fluorescent characteristics of AGEs.
The AGE-reader is a desktop unit on which the patient positions the volar side of the right lower arm on a light source. Accumulation of skin advanced glycation end products is measured by skin autofluorescence (SAF). The measurements of SAF are validated against AGE-levels in skin biopsies in healthy controls, in patients with diabetes and in patients on hemodialysis[39, 40].

The AGE-reader measures skin auto-fluorescence as a measure of accumulation of AGEs in the skin

AGEs are thought to have a role in the concept of "metabolic memory". The concept of "metabolic memory" refers to a long-lasting detrimental effect on tissues of longstanding hyperglycemia continuing after improvement of glycemic control. This was shown in the Diabetes Control and Complications Trial and the Epidemiology of Diabetes Interventions and Complications (DCCT-EDIC), in which complication rate was lower in the original intensive treatment group not only at the time of intensive control, but also in the follow-up period when mean glycemic control was comparable to that in the original less controlled comparator group[41]. Similar results were shown from follow-up of the UKPDS[42]. AGEs can provide information about hyperglycemia for a much longer period than HbA1c and could therefore be a better reflection of "metabolic memory". Furthermore AGE-accumulation can also play a pathophysiological role, as shown in experimental studies, although this could not be convincingly confirmed in intervention studies in humans[43].
Since the association of AGE accumulation with diabetes complications has been investigated in a number of studies, we first aimed to review all studies

using the non-invasive AGE-reader (Chapter 6). Subsequently we assessed these associations for different complications in our own cohort of type 2 diabetes patients (Chapter 7). AGE accumulation has never been investigated in relation to gestational diabetes mellitus, therefore we also investigated its possible use in this specific type of diabetes.

Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is carbohydrate intolerance first discovered during pregnancy. Patients are diagnosed with GDM by oral glucose tolerance test (OGTT). OGTT is performed in women with maternal risk factors or in women with signs or symptoms of hyperglycemia including intra-uterine growth acceleration. GDM is considered to be a mild form of diabetes. Nevertheless, GDM is associated with an increased incidence of maternal and fetal/neonatal complications[44]. Patients that show elevated glucose levels on OGTT are treated by diet and if necessary insulin treatment. Recent intervention trials have shown that treatment of hyperglycemia improves pregnancy outcome[45, 46]. GDM is a major risk factor for development of type 2 diabetes in later life[47]. Apparently patients with a susceptibility for type 2 diabetes are prone to develop GDM.
OGTT is an invasive, laborious test. If AGE-accumulation is present in GDM patients the non-invasive AGE-reader could possibly be used to detect GDM. As a first step to evaluate if the AGE-reader could be used to detect GDM we evaluated if AGE-accumulation, measured as skin autofluorescence (SAF) was present in GDM patients (Chapter 8). We also investigated if SAF changed during normal pregnancy or pregnancy complicated by diabetes and if it is associated with adverse pregnancy outcome (Chapter 9).

Overall aim and objectives:

The overall aim of this thesis is to investigate the usefulness of other parameters of glycemic control, glycemic variability (showing actual glucose levels) and advanced glycation end products (a more long-term biomarker of mean glucose), in the treatment of patients with diabetes. Specifically the following objectives will be addressed:

Glycemic variability:

To investigate which parameter for glycemic variability could be used in clinical practice and if progression of glycemic variability differs between type 1 and type 2 diabetes (Chapter 2).

To investigate the clinical relevance of glycemic variability, by exploring whether increased glycemic variability is associated with worse quality of life (Chapter 3).
To investigate methods or techniques to reduce glycemic variability, such as addition of a DPP4-inhibitor to existing insulin regimen or with real-time-continues glucose monitoring (Chapter 4 and 5).

Advanced glycation end products:

To review and investigate the association of accumulation of skin AGEs with diabetes complications (Chapter 6 and 7).
To explore the usefulness of the AGE-reader to detect gestational diabetes mellitus and to detect patients at risk for obstetric complications (Chapter 8 and 9).

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Chapter 2

Glycemic Variability in Inadequately Controlled Type 1 Diabetes and Type 2 Diabetes on Intensive Insulin Therapy, a Cross-sectional, Observational Study

W. L. Greven, J. W.J. Beulens, D. H. Biesma, S. Faiz, H. W. de Valk

Diabetes Technol Ther 2010; 12(9):695-699.

Abstract

Background

Glycemic variability is suggested to be a predictor for the risk of complications of diabetes. A multitude of parameters to express glycemic variability have been described, but no gold standard exists. The easy measurable parameter SD has been shown to be strongly related to other parameters in a group of patients with mostly well-controlled type 1 and type 2 diabetes. Glycemic variability is higher in type 1 diabetes compared with type 2 diabetes in mixed populations with different treatments, but studies in patients on intensive insulin treatment are lacking. Therefore in this study we investigate different parameters of glycemic variability and differences between type 1 and type 2 diabetes in inadequately controlled patients on intensive insulin treatment.

Methods

In this cross-sectional, observational study we describe glycemic variability, measured as SD, coefficient of variation, continuous overall net glycemic action, and mean of daily differences in a cohort of inadequately controlled type 1 diabetes (n = 166) and type 2 diabetes (n = 58) patients on intensive insulin treatment.

Results

SD of 48h (SD_{total}) was highly correlated to all other measured parameters of glycemic variability (r = 0.66–0.88). All parameters of glycemic variability were significantly higher in type 1 diabetes, compared to type 2 diabetes ($P < 0.001$), although hemoglobin A1c and mean glucose were comparable and treatment regimen was the same. In the cohort of type 2 diabetes patients but not type 1 diabetes, a longer duration of insulin therapy was associated with higher glycemic variability.

Conclusions

SD_{total} is a conveniently measurable parameter to express glycemic variability in patients with inadequate control with intensive insulin therapy. Patients with type 1 diabetes and long-lasting type 2 diabetes have the highest glycemic variability.

Introduction

Hyperglycemia is a well-established risk factor for complications in type 1[1] and type 2[2] diabetes. However, glycemic variability is also suggested to be a predictor for the risk of complications of diabetes,[3] but its role continues to be debated[4]. Glycemic variability can be calculated from data obtained by continuous glucose monitoring (CGM). A multitude of parameters to express glycemic variability have been described, but no gold standard exists. The easy measurable parameter SD has been shown to be strongly related to other parameters of glycemic variability in a group of patients with mostly well-controlled type 1 and type 2 diabetes[5].

Glycemic variability is higher in type 1 diabetes compared with type 2 diabetes in mixed populations with different treatments,[6,7] but studies in patients on intensive insulin treatment are lacking.

The aims of the present study were to investigate whether SD was correlated to other parameters of glycemic variability in inadequately controlled patients on intensive insulin treatment. Furthermore, our aim was to investigate whether glycemic variability is higher in type 1 diabetes versus type 2 diabetes, as well as the relation between glycemic variability and different factors in both type 1 and type 2 diabetes.

Subjects and Methods

Patients

Data (CGM, clinical, and laboratory data) from all patients receiving off-line CGM (CGMS® System Gold™, Medtronic MiniMed, Northridge, CA) from November 2003 until July 2009 were used. CGM was ordered by the treating physician because of inadequate glycemic control (variable glucose values, hypoglycemia episodes, inadequate hemoglobin A1c [HbA1c], or other reasons). Usually a combination of different reasons was present to order CGM for inadequate control. If patients received CGM multiple times in this period, only the first recording was used. The first two fully recorded days were used for analysis, and patients with more than two missing values were excluded. Among a total of 329 patients who received CGM, 39 recordings were incomplete and therefore excluded. The 290 remaining data were from patients with type 1 diabetes (n = 174), type 2 diabetes (n = 65), secondary diabetes due to cystic fibrosis (n = 27), and other types of diabetes (prednisone-induced [n = 5], latent autoimmune diabetes in adults [n = 7], post-pancreatic surgery [n = 2], and not specified [n = 10]). For this study we included patients with type 1 diabetes (n = 166) or

patients with type 2 diabetes ($n = 58$) on intensive insulin therapy (insulin pump [continuous subcutaneous insulin infusion] or multiple daily injections). Clinical data, (age, sex, body mass index [BMI], HbA1c, type of therapy, duration of disease, and duration of insulin treatment) were retrieved from medical records.

Analysis of continuous glucose measurements

Data from CGM were recorded for approximately 72 h. Data from the first two fully recorded days (from 00:00 day 1 until 24:00 day 2) were used. SD for both days was calculated. SD was subdivided in SD_{total} (SD of 48 h), SD_{day1}, and SD_{day2}. Mean and percentage coefficient of variation (%CV) were calculated for both days separately. Furthermore, the mean %CV of these two days was calculated. Continuous overall net glycemic action (CONGA) and mean of daily differences (MODD) were calculated as described earlier by McDonnell et al[8]. $CONGA_1$, $CONGA_2$, and $CONGA_4$ were calculated (SD of the differences between an observation and another observation 1, 2, or 4 h before, respectively). $CONGA_{1-4}$ values for both days were calculated separately, and the mean CONGA for both days was also calculated.

Statistical analysis

Results are presented as mean and SD values. Correlations between parameters of glycemic variability are expressed as Pearson's correlations coefficient. A Student's *t* test was used to compare between any two groups. Difference in variability between type 1 and type 2 diabetes were adjusted for different factors (age, sex, BMI, HbA1c, type of therapy, duration of disease, and duration of insulin therapy), using linear regression. Level of significance was set at $p < 0.05$. Linear regression models (univariate and multivariate) were used to investigate relations between glycemic variability and different factors (as mentioned above). Because type 1 diabetes and type 2 diabetes are different diseases, we analyzed these groups separately. The relation of each factor with glycemic variability was investigated separately in univariate models, and subsequently all factors with a p value of < 0.10 were investigated in the multivariate model.

Results

For the group of type 1 diabetes or type 2 diabetes baseline characteristics are given in *Table 1*. Age and BMI are significantly higher in type 2 diabetes, duration of diabetes and the duration of insulin therapy are significantly longer in type 1 diabetes, but HbA1c was comparable in both groups. In type

1 diabetes more patients were receiving insulin pump therapy. Hypoglycemia, measured by CGMS Gold (glucose <3.9 mmol/L), occurred more frequently in type 1 diabetes, whereas measured hyperglycemia (glucose >10.0 mmol/L) was comparable in both groups (*Table 1*).

Table 1. Group characteristics of patients with type 1 and type 2 diabetes

	Type 1 diabetes	Type 2 diabetes
Number	166	58
Age (years)	41.4 (11.5)*	55.3 (12.8)*
% female	55.4%	43.1%
BMI	24.9 (3.4)*	31.3 (6.1)*
HbA1c (%)	8.3 (1.3)	8.5 (1.2)
% on pump therapy	35.5%*	17.2%*
Duration (years) of		
Diabetes	21.3 (12.3)*	14.1 (8.3)*
Insulin therapy	21.3 (12.4)*	8.7 (5.9)*
Time in (%)		
Hypoglycemia	9.3 (10.2)*	3.9 (6.4)*
Hyperglycemia	37.8 (22.3)	40.6 (26.5)

Data are presented as mean (SD) or percentage values.
*P < 0.01. BMI = body mass index; HbA1c = hemoglobin A1c

SD_{total} was highly correlated ($p < 0.001$) with all other parameters on both days and with the MODD. Data shown in *Table 2* are from mean values of 2 days from all parameters and MODD. $CONGA_1$, $CONGA_2$, and $CONGA_4$ correlated with each other on the same day, as well as between days individually.

Parameters of glycemic variability were compared between type 1 diabetes and type 2 diabetes. SD_{total}, MODD, %CV, $CONGA_1$, $CONGA_2$, and $CONGA_4$ (mean of 2 days) were all significantly higher in type 1 diabetes compared to type 2 diabetes ($p < 0.001$), whereas the mean glucose and HbA1c were comparable (*Table 3*). When adjusted for age, BMI, sex, HbA1c, type of therapy, and duration of disease or insulin use, these results did not change.

Table 2. Correlations between total SD over a 48-h period and different parameters of glycemic variability

	SD_{total}
$\%CV_{mean}$	0.66**
Mean	
$CONGA_1$	0.76**
$CONGA_2$	0.82**
$CONGA_4$	0.88**
MODD	0.79**

Pearson's correlation coefficient R is given for parameters of glycemic control. Mean values are those for days 1 and 2. **Correlation is significant at the level $p < 0.01$.
CONGA1, CONGA2, and CONGA4 = continuous overall net glycemic action (between an observation and another observation 1, 2, or 4 h before, respectively); %CV = percentage coefficient of variation; MODD = mean of daily differences; SD_{total} = SD of a 48-h period.

Table 3. Parameters of glycemic control and glycemic variability in type 1 versus type 2 diabetes mellitus

	Type 1 diabetes	**Type 2 diabetes**	**P value**
Glycemic control			
Mean glucose (mmol/L)	9.2 (2.4)	9.5 (2.3)	NS
HbA1c (%)	8.3 (1.3)	8.5 (1.2)	NS
Glycemic variability			
SD_{total}	3.6 (1.1)	2.7 (0.7)	< 0.001
$\%CV_{mean}$	0.4 (0.1)	0.3 (0.1)	< 0.001
Mean			
$CONGA_1$	2.5 (0.7)	1.9 (0.5)	< 0.001
$CONGA_2$	3.7 (1.1)	2.7 (0.7)	< 0.001
$CONGA_4$	4.6 (1.5)	3.3 (1.0)	< 0.001
MODD	4.0 (1.5)	2.9 (1.1)	< 0.001

Data are mean (SD) values.
CONGA1, CONGA2, and CONGA4 = continuous overall net glycemic action (between an observation and another observation 1, 2, or 4 h before, respectively); %CV = percentage coefficient of variation; HbA1c = hemoglobin A1c; MODD = mean of daily differences; NS = not significant; SD_{total} = SD of a 48-h period

In a univariate linear regression model for glycemic variability in type 1 diabetes only HbA1c and type of therapy (pump vs. MDI) were related to glycemic variability, whereas age, BMI, and duration of disease or duration of insulin therapy were not (*Table 4*). In a multivariate model, both factors were independently related to variability, but HbA1c was the strongest factor.
In a univariate linear regression model for glycemic variability in type 2 diabetes, only duration of insulin therapy was related to glycemic variability,

whereas age, BMI, HbA1c, type of therapy, and duration of disease were not related to glycemic variability, although for HbA1c a trend was shown. In a multivariate model, including both duration of insulin and HbA1c, both factors were independently related to variability, although duration of insulin therapy was the strongest factor.

In type 2 diabetes, every year of insulin use raises the SD_{total} by 0.044, so when a patient uses 10 years of insulin his SD_{total} could rise 0.44. The difference in mean SD_{total} between type 1 diabetes and type 2 diabetes is 0.924. One can therefore calculate that only after 21 years of insulin treatment mean SD_{total} in type 2 diabetes will be similar to the mean SD_{total} in type 1 diabetes.

Data provided are for glycemic variability, as measured by SD_{total}. However, using other parameters of glycemic variability similar results were obtained (data not shown).

Figure 1 shows a scatterplot for the relation between duration of insulin therapy and SD_{total}. As can be seen from the horizontal line in *Figure 1*, SD_{total} in type 1 diabetes does not change at all with duration of insulin therapy (this is in concordance with the ß from *Table 4*.

Table 4. Relation of different parameters with glycemic variability (total SD over a 48-h period)

	Type 1 diabetes		Type 2 diabetes	
	Univariate	**Multivariate**	**Univariate**	**Multivariate**
Age	0.005 (P = 0.46)		0.007 (P = 0.33)	
BMI	-0.016 (P = 0.59)		-0.018 (P = 0.31)	
HbA1c	0.321 (P < 0.001)**	0.318 (P < 0.001)**	0.136 (P = 0.089)	0.175 (P = 0.035)*
Pump therapy	-0.31 (P = 0.036)*	-0.367 (P = 0.025)*	-0.026 (P = 0.92)	
Duration of diabetes	0.001 (P = 0.93)		0.013 (P = 0.27)	
Duration of insulin therapy	0.001 (P = 0.91)		0.036 (P = 0.042) *	0.044 (P = 0.014)*

Relations are expressed as β (P value). *Association is significant at the 0.05 level. **Association is significant at the 0.01 level. BMI = body mass index; HbA1c = hemoglobin A1c

As can be concluded from *Figure 1*, in type 2 diabetes SD_{total} rises with a longer duration of insulin therapy (which is also in concordance with results in *Table 4*). The difference in interception point of the two lines can be explained by the fact that *Figure 1* shows a univariate analysis of the relation of SD_{total} with duration of insulin therapy, whereas *Table 4* considers a multivariate analysis, taking into account the effect of HbA1c.

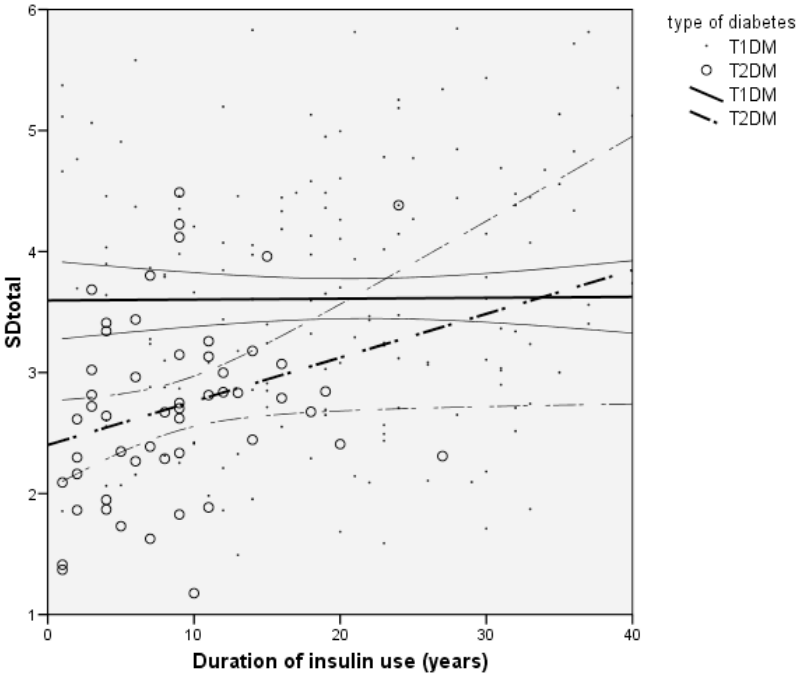

Figure 1. Scattergram with superimposed regression lines (and 95% confidence interval) showing the relation of glycemic variability (SD over a 48-h period [SD_{total}]) with duration of insulin therapy in type 1 and type 2 diabetes

Discussion

This study shows higher glycemic variability in type 1 diabetes patients compared to type 2 diabetes patients, whereas mean glucose concentration, HbA1c levels, and treatment regimens were similar. In type 2 diabetes but not type 1 diabetes patients, a longer duration of insulin therapy was related to higher glycemic variability. Finally, SD_{total} in inadequately controlled type 1

and type 2 diabetes was highly correlated with other parameters of glycemic control and can be used as an easily accessible measure of variability.

More than 20 parameters have been described to measure glycemic variability, but no gold standard exists[5]. Although mean amplitude of glucose excursions is the oldest parameter to measure glycemic variability, it is open to several interpretations[9]. Rodbard[5] described a strong correlation between SD_{total} and other parameters of glycemic variability in a mixed patient population, with mostly adequate control. Our findings in inadequately controlled patients are in concordance with this study, and correlations were comparable in strength[5,10]. Because data during the first few hours could be unreliable, these are not included in the calculations. A fixed amount of data (48 h) per patient is used to calculate SD_{total}.

Higher glycemic variability in type 1 diabetes versus type 2 diabetes has been described previously. One study showed higher variability (measured as SD) in type 1 diabetes versus type 2 diabetes, but information on treatment or HbA1c was lacking[6]. Another study also found a higher variability (measured as mean amplitude of glucose excursions) in type 1 diabetes versus type 2 diabetes, although treatment of type 1 diabetes (all intensive insulin) was different from treatment in type 2 diabetes (mix of oral agents/ nonintensive/ intensive insulin treatment)[7]. In our study, similar differences in variability cannot be explained by differences in treatment or HbA1c because both groups were receiving intensive insulin treatment and had similar HbA1c concentrations, but it is more likely to be caused by the type of diabetes per se.

Interestingly, duration of insulin therapy was related to glycemic variability in type 2 diabetes but not in type 1 diabetes. This could be explained by a quick progression of disease in type 1 diabetes. With progressive disease, ß-cell mass and endogenous insulin decrease. This is followed by α-cell dysfunction, which leads to inappropriate hyperglucagonemia after meals. In type 1 diabetes this process occurs quickly, leaving little residual endogenous insulin secretion in a very short time. In type 2 diabetes, progression of disease occurs much slower, and endogenous insulin secretion will slowly decrease over many years, which may explain the relationship between the duration of insulin use and the variability in type 2 diabetes. Despite this, no relation was found between duration of disease and variability. This may be explained by the fact that, in contrast to the start of insulin, the start of type 2 diabetes is difficult to pinpoint because a variable silent period can be present before detection.

The suggested relation between ß-cell loss (duration of insulin therapy) and variability in type 2 diabetes is in concordance with other studies. One study showed that glycemic variability was related to postprandial ß-cell dysfunction in type 2 diabetes patients on oral agents or diet[11]. Another study also found a relation between insulin duration and variability[12].

Because glycemic variability is suggested to be a risk factor for complications, pharmacological agents influencing variability could be of interest in the future. Glucagon-like peptide-1 analogs as well as dipeptidyl peptidase-4 inhibitors have already been shown to decrease glucose variability[13,14]. Because glycemic variability is higher in type 1 diabetes and increases with duration of insulin use in type 2 diabetes, these agents might be of specific interest in these patient groups.

This study has some limitations. Although all patients who received CGM in a particular period in our center were included in this study, CGM was performed for clinical reasons and not at random. So results can only be extrapolated to patients in daily clinical practice who need CGM. Furthermore, although all patients attended our clinic and all patients received standard care, food intake and insulin regimens were not specifically standardized on the days patients wore the CGM device.

In conclusion, SD_{total} is a convenient parameter for the expression of glycemic variability in patients with inadequate control with intensive insulin therapy. Patients with type 1 diabetes and long-lasting type 2 diabetes have the highest glycemic variability. In type 2 diabetes, but not type 1 diabetes longer duration of insulin use is associated with increased glycemic variability.

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Chapter 3

Is Higher Glycemic Variability Associated with Reduced Quality of Life in Type 2 Diabetes Patients?

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(Submitted)

Abstract

Background

Patients with diabetes have a reduced quality of life, especially those with elevated Hba1c. This study hypothesizes that high glycemic variability also negatively influences quality of life.

Methods

In this cross-sectional observational study glycemic variability was measured in 124 patients with type 2 diabetes by standard deviation of glucose data from 48 hours continuous glucose monitoring. Quality of life was assessed with different questionnaires: Problem Areas in Diabetes Scale, 12-item short form general health survey and EuroQaulity of life -5 dimensions visual analogue scale. Association of glycemic variability and quality of life was analysed in the entire study population, and in pre-defined subgroups (insulin treatment (n=68); other treatment (n=56)).

Results

A higher glycemic variability tended to be associated (p=0.07) with a worse PAID score in the entire study population (β 0.20(95%CI -0-01-0.42)), but this attenuated when adjusted for confounders. Higher glycemic variability was associated with worse PAID scores in insulin-treated patients (0.33(0.01-0.65)), especially in the domains of the PAID questionnaire "treatment-related problems" (0.29(0.09-0.50)) and "diabetes-related emotional problems" (0.32(0.03-0.61)). The associations for these specific domains attenuated (0.24 (0.04-0.44); 0.24(-0.06-0.54)) after correction for confounders and after correction for HbA1c (0.15(-0.07-0.38); 0.17(-0.17-0.52)). Glycemic variability was not consistently associated with parameters from other questionnaires.

Conclusion

High glycemic variability was associated with reduced quality of life, albeit not independent of HbA1c. Since this association was found in insulin-treated type 2 diabetes patients only; glycemic variability could be a potential treatment goal for this particular group.

Background

Patients with diabetes mellitus have a decreased quality of life as compared to the general population, especially when diabetes complications and hypoglycemia are present[1-4]. In addition, poor glycemic control (as measured by elevated HbA1c) is associated with worse quality of life in most studies[1, 5-7]. One study suggests that the relation between HbA1c and quality of life is not strictly linear, but that quality of life is best in patients with HbA1c levels between 53 and 64 mmol/mol [1].

Increased glycemic variability is an additional characteristic of the diabetic state. While glycemic variability and its possible association with diabetes complications has been researched thoroughly. This association remains controversial because studies on this topic showed inconsistent results[8-14]. In this study we aim to investigate whether glycemic variability is associated with reduced quality of life. Such an association would emphasize the importance of glycemic variability as a clinical problem.

Our hypothesis is that increased glycemic variability might lead to non-predictability of the disease, a decrease in perceived ability to control it and in dissatisfaction with treatment. With large glycemic variability the daily effort to approximate a non-diabetic metabolic state can seem futile and patients can feel resigned, which can again lead non-optimal treatment and self-management. This might contribute to diabetes-related emotional problems and patients might experience more treatment-related problems and hence reduced quality of life.

In a previous small study in type 1 diabetes patients (n=32) only an association between mood and actual level of glucose (measured real-time) was found, but no association of mood with glycemic variability in the previous hour[15]. In contrast, two prior cross-sectional studies in type 2 diabetes had reported an association between reduced glycemic variability and improved health-related quality of life[16, 17]. However, both studies investigated only a small sample of patients (n=23 – 54) and neither corrected for HbA1c.

In our study, we aim to investigate this association in a large sample of type 2 diabetes patients and determine whether or not glycemic variability is associated with quality of life, independent of HbA1c.

Materials and Methods

Study design and patients

This observational prospective study was conducted at the University Medical Centre Utrecht (UMCU) in the Netherlands. All patients in this study were participating in a nationwide long-term biobank initiative for patients with type 2 diabetes[18]. The current study was a local add-on to this national database study. It was conducted according to Good Clinical Practice and the protocol was approved by the ethics committee of the University Medical Centre Utrecht. Type 2 diabetes patients attending the (outpatient) clinic of the UMCU were approached to participate in this study. Written informed consent was obtained from all patients.

Assessment of glycemic variability

Patients wore an off-line continuous glucose monitoring system for > 48 hours. With this device glucose levels were recorded subcutaneously every 5 minutes, and all data were blinded for the patients. The first 48 hours from 00.00 – 24.00 were used to calculate glycemic variability. Glycemic variability was calculated as the SD of all glucose data from 48 hours. The glucose data were complete (576 glucose data) for 81 % of the patients. Patients with less than 50% recorded glucose data (<288 readings) were defined as inadequate data and were excluded from the analysis (n=2).

Quality of life questionnaires

Several questionnaires were used to assess quality of life. First, the Dutch version of the Problem Areas in Diabetes Scale (PAID) questionnaire was used to measure diabetes-specific quality of life[6]. The questionnaire consists of 20 items, which are scored by the patient on a 5 point Likert scale (0= no problem, 4= severe problem). The total score is then amplified by 1.25, rendering a score from 0 – 100 [19] with 0 being the best achievable score and 100 the worst score. The PAID score is known to have consistently high internal reliability (α=0.90) and a sound test-retest reliability in stable patients (r=0.83)[19]. The questionnaire can be divided into four subdomains: diabetes-related emotional problems, treatment-related problems, food-related problems and social support-related problems [6].
Furthermore, two generic measures of quality of life were used. The Dutch version of the 12-item short form general health survey (SF-12) was used to assess health-related quality of life[20, 21]. The SF-12 contains 12 items derived from the more extensive SF-36 HRQOL form. This questionnaire is reported as

two separate scores for quality of life, considering a physical component score and a mental component score. Scores range from 0 to 100, with 100 being the best quality of life achievable and 0 the worst. Second, the EuroQuality of life -5 dimensions visual analogue scale (EQ5D-VAS) was used. This questionnaire consists of an overall quality of life score on a visual analogue scale (VAS) from 0 (worst) to 100 (best), on which a patient estimates his or her own health state. A list of five questions within separate dimensions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) is added to this VAS. Patients can score 1 indicating no problem, 2 indicating some problems or 3 indicating extreme problems. A health state can be derived from the answers to the questions, which can be further translated into a single index value.

Statistical analysis

Results are presented as mean and SD for normally-distributed variables and median and range for non-normally distributed parameters. A student's t-test was used to compare between two groups with normal distribution. Linear regression analysis was used to identify parameters associated with outcome of interest. If the variables were non-normally (right-skewed) distributed, then they were log-transformed and in case of left-skewed distribution, they were exponentially transformed. Regression analyses were corrected for pre-defined confounders; age, sex, duration of diabetes, educational level or use of antidepressant drugs and corrected for HbA1c in a separate model. Missing values of confounders were imputed, using the mean value. Association of glycemic variability with quality of life was analysed in the whole group, and in pre-defined subgroups based on treatment regimen (insulin treatment (n=68) versus other treatment (n=56). These subgroups had been predefined because glycemic variability was expected to be higher in insulin users than in non-insulin users. The interaction between glycemic variability and treatment group was tested by including an interaction term in the model. Standardised β's were used to compare the associations of HbA1c and SD with outcome (scores on PAID questionnaire) separately. The different domains of the PAID score were also analysed as an additional outcome. Because the distribution of residuals from linear regression for the domains "problems with food" and "lack of social support" was not entirely normal, these analyses were repeated using logistic regression (after dichotomizing data below or above mean). Finally, a sensitivity analysis was performed on all analyses to adjust for selection bias due to patients not consenting separately to continuous glucose monitoring using inverse probability weighting. Level of significance was set at $p< 0.05$.

Results

From February 2010 till July 2013, 1372 patients were screened for this national biobank study and 508 patients were included, 5 withdrew consent, leaving 503 patients for analysis. Continuous glucose monitoring data were not available in all 503 patients since an additional informed consent was necessary for wearing the device and the majority of the patients only consented to questionnaires and laboratory measurements. A total of 126 patients signed the additional consent for continuous glucose monitoring and from these patients 124 (98%) had adequate data and could be included for analysis in this study.

Baseline characteristics are shown in table 1. The mean SD was 2.2 (1.0), and this was significantly higher in patients on intensive insulin scheme (2.7) compared to patients on oral agents or diet only (1.7, p<0.001). HbA1c was associated with SD (Pearson coefficient r= 0.46 p=<0.001) and with the score on the PAID questionnaire (r=0.30, p=00.1), but not with the other parameters of quality of life. Included patients were predominantly of Caucasian origin, and had a median age was of 58 years, BMI 32, and HbA1c 58. Duration of diabetes was approximately 11 years and most patients (55%) used insulin. Baseline characteristics of included patients were compared to characteristics of patients that did not consent to continuous glucose monitoring. The latter were older, had a longer duration of diabetes and scored better on the PAID and SF 12 mental component score (*Table 1*).

A higher glycemic variability tended to be associated (p=0.067) with reduced PAID score (β 0.2; 95%CI -0.014 – 0.41). The association attenuated after correction for pre-defined confounders (age, sex, duration of diabetes, educational level or use of antidepressant drugs) (0.17 (-0.06-0.40) p=0.158) and after adjusting for HbA1c (0.03 (-0.24-0.30) p=0.817) (*Table 2*).

The association of glycemic variability with the PAID score was also analyzed in pre-defined subgroups based on treatment regimen (interaction term for SD * treatment p=0.14). A higher glycemic variability was associated with a worse PAID score in the insulin-treated patients only (0.33 (CI 0.01 – 0.65), p = 0.042), whereas variability was not related to quality of life among patients on other treatment. The association in the insulin-treated patients attenuated to non-significance after correction for confounders (0.26 (-0.09-0.61) p=0.139) and adjustment for HbA1c (0.20 (-0.20-0.59) p=0.316) (*Table 2*).

Table 1 Baseline characteristics of the 124 type 2 diabetes patients included, compared to 377 patients, who did not consent to continuous glucose monitoring (CGM)

	Patients included N=124	**Patients that did not consent to CGM** N=377
Age	58 (11)	61 (12) *
Sex (% female)	46 %	38%
Ethnicity (% caucasian)	90 %	85%
BMI	32 (6)	31 (6)
HbA1c	58 (18)	58 (14)
Duration of DM	11 (8)	13 (10) *
Blood pressure (MAP in mmHg)	98 (10)	97 (12)
Current smoking	20 %	16%
Use of antidepressant drugs	6%	9%
Complications		
microvascular		
neuropathy	26 %	30%
retinopathy	20 %	25%
nephropathy	19 %	20%
macrovascular	32 %	38%
Therapy		
None	5 %	5%
Oral agents only/ GLP-1 analoga	40 %	39%
Insulin	55 %	56%
Long-acting	6 %	6%
Premix	4 %	8%
Intensive	38 %	35%
CSII	7 %	7%
Glycemic variability (SD)	2.2 (1.0)	Not determined
Quality of life		
PAID	12.5 (0-75)	7.5 (0-67.5) *
SF-12		
Physical component score	39 (11)	41 (11)
Mental component score	46 (10)	48 (10) *
EQ5D		
VAS score	64 (18)	65 (19)
Index Value	0.78 (-0.13 – 1.00)	0.81 (-0.26 – 1.00)

Results are described as mean (SD) or median (range) or percentage
Neuropathy is defined as a score on the neurothesiometer of > 25 V
Retinopathy is defined as any degree of retinopathy (as assessed by fundus photographs)
Nephropathy is defined as GFR (MDRD) < 60. Macro-vascular complication is defined as a composite endpoint (MI or CVA or angioplasty or vascular surgery (coronary, carotid, femoral, iliacal, aortal).
Antidepressants drugs were defined as drugs that are indicated for depression, however drugs commonly prescribed for neuropathic pain were excluded (amitriptyline, duloxetine, nortrilen)
* Significant difference ($p < 0.05$)

Table 2 Linear regression analysis showing association of glycemic variability (SD) with (log)PAID score in patients on different therapeutic regimen

	Total group		Diet or oral agents only		Insulin treatment	
	β (CI)	P	β (CI)	P	β (CI)	P
SD	0.20 (-0.01–0.42)	0.067	-0.03 (-0.42–0.35)	0.858	0.33 (0.01 - 0.65)	0.042
MV	0.16 (-0.07–0.38)	0.167	-0.02 (-0.41-0.38)	0.926	0.26 (-0.07 – 0.59)	0.119
MV + HbA1c	0.00 (-0.26-0.26)	0.977	-0.28 (-0.71–0.15)	0.201	0.17 (-0.21 – 0.54)	0.371

PAID = Problem Areas in Diabetes Scale; MV = adjusted for pre-defined covariates (age, sex, duration of diabetes, use of antidepressant drugs and education level); MV + HbA1c = also adjusted for HbA1c

Table 3 Linear regression analysis showing association of glycemic variability (SD) with (log)PAID score in different pre-defined domains of the PAID questionnaire

	Total group		Diet or oral agents only		Insulin treatment	
	β (CI)	P	β (CI)	P	β (CI)	P
Domains of PAID						
Diabetes-related emotional problems	0.23 (0.04-0.43)	0.021	-0.02 (-0.38-0.35)	0.934	0.32 (0.03-0.61)	0.031
MV	0.18 (-0.03-0.38)	0.096	0.00 (-0.38-0.38)	0.995	0.24 (-0.06-0.54)	0.108
MV + HbA1c	0.04 (-0.21-0.28)	0.754	-0.21 (-0.63-0.21)	0.313	0.17 (-0.17-0.52)	0.320
Treatment-related problems	0.23 (0.09-0.37)	0.001	0.15 (-0.09-0.40)	0.211	0.29 (0.09-0.50)	0.006
MV	0.22 (0.08-0.37)	0.003	0.15 (-0.12-0.43)	0.270	0.24 (0.04-0.44)	0.021
MV + HbA1c	0.11 (-0.06-0.27)	0.211	-0.02 (-0.32-0.27)	0.943	0.15 (-0.07-0.38)	0.171
Food-related problems	-0.01 (-0.15-0.13)	0.879	-0.05 (-0.28-0.19)	0.684	0.07 (-0.14-0.28)	0.504
MV	-0.03 (-0.18-0.11)	0.639	-0.05 (-0.30-0.20)	0.694	0.01 (-0.21-0.22)	0.935
MV + HbA1c	-0.12 (-0.29-0.04)	0.144	-0.18 (-0.46-0.10)	0.201	-0.06 (-0.32-0.19)	0.617
Social support-related problems	0.05 (-0.07-0.16)	0.410	-0.12 (-0.28-0.04)	0.151	0.09 (-0.10-0.27)	0.366
MV	0.03 (-0.09-0.14)	0.628	-0.11 (-0.28-0.07)	0.226	0.03 (-0.16-0.22)	0.740
MV + HbA1c	-0.03 (-0.17-0.11)	0.677	-0.14 (-0.34-0.06)	0.165	-0.02 (-0.24-0.20)	0.850

PAID = Problem Areas in Diabetes Scale; MV = adjusted for pre-defined covariates (age, sex, duration of diabetes, use of antidepressant drugs and education level); MV + HbA1c = also adjusted for HbA1c

Glycemic variability was associated with two of the four domains of the PAID score ("treatment-related problems" and "diabetes-related emotional problems"). These associations were found in the total and in the insulin-treated group, but not in the subgroup on oral treatment or diet (*Table 3*). The associations were significant after correction for confounders in the domain "treatment-related problems" domain (0.24 (0.04-0.44) p=0.021), but attenuated in the domain "diabetes-related emotional problems" (0.24 (-0.06-0.54) p=0.108). None of the associations were significant after correction for HbA1c (0.15 (-0.07-0.38) p=0.171, 0.17 (-0.17-0.52) p=0.320) data for insulin-treated patients)). When analyses were repeated with logistic regression analysis, results were comparable.

Comparison of the standardized β's for association of HbA1c or SD with PAID scores showed that these were comparable in the insulin-treated group (SD β 0.26 p=0.042; HbA1c β 0.27 p=0.034 (univariate) SD β 0.13 p=0.371; HbA1c β 0.15 p=0.331 (multivariate)), while in the total group (SD β 0.17 p = 0.067; HbA1c β 0.26 p<0.001) and other treatment group (SD β -0.03 p=0.858; HbA1c β 0.32 p=0.017) HbA1c was the most important or even only factor (multivariate HbA1c β 0.25-0.38, SD NS).

Glycemic variability was not associated with scores on generic measures of health-related quality of life (SF12 or EQ5D) with the exception of a significant association between higher glycemic variability and worse scores on SF12 physical component score in the insulin-treated group (-3.29 (-6.42- -0.17), p =0.039) after correction for confounders and HbA1c, while the opposite was found in patients with other treatment for the SF12 mental component score (3.52 (0.08-6.96) p=0.045) (Suppl 1).

Sensitivity analyses to adjust for selection bias due to patients that did not consent separately to continuous glucose monitoring using inverse probability weighting showed comparable results. The association between glycemic variability and PAID score in the entire study population (β 0.20, p=0.067) was comparable after sensitivity analysis (0.20, p=0.053); the association was also comparable in the insulin-treated group (β 0.33 p=0.042, after inverse probability weighting 0.31, p=0.043).

Discussion

This study shows that increased glycemic variability is associated with reduced quality of life, measured by a diabetes-specific questionnaire, in insulin-treated patients with type 2 diabetes only. These associations were present in the sub-domains considering diabetes-related emotional problems and treatment-related problems, but they were not independent from HbA1c.

Our findings are in concordance with two previous smaller studies in type 2 diabetes patients, which also showed an association between higher glycemic variability and reduced health-related quality of life[16, 17]. However these studies did not correct for HbA1c. We found that the association of glycemic variability with diabetes-related quality of life attenuated to non-significant when corrected for HbA1c. Therefore, our data suggest that the association of glycemic variability with worse quality of life is not independent from HbA1c.

Associations of higher glycemic variability with quality of life were only found in the insulin-treated patients. This was expected since this particular group has a higher glycemic variability. The strength of associations of HbA1c or SD with PAID scores were comparable in the insulin-treated group, while in the group with patients on other treatment, HbA1c was the most important factor. These results suggest that glycemic variability is of interest as a potential clinical treatment goal in insulin-treated patients especially.

Associations of glycemic variability with quality of life were mainly found with scores on the PAID questionnaire. This was expected since the PAID questionnaire is a disease-specific questionnaire, consisting of questions that could be specifically affected by glycemic variability. We hypothesized that a high degree of glycemic variability can lead to more problems with the manageability and controllability and thus treatment of the disease. Moreover, the daily effort to approximate normal glucose levels can seem futile to patients with large glycemic variability, which can result in frustration and negative feelings about diabetes. Our results are in line with this hypothesis since we observed associations with the domains "treatment-related problems" and "diabetes-related emotional problems" particularly. However, we have to consider the possibility that it could also be the other way around, that patients with better quality of life are more capable of adequate self-management and hence experience less glycemic variability.

We did not find associations of glycemic variability with scores on health-related questionnaires, not specifically designed for diabetes patients (SF12, EQ5D), with the exception of the corrected positive association between variability and SF12 physical component score in the insulin-treated group, while the opposite was found for the mental component score in the non-insulin-treated patients. This is in contrast to the analysis with the PAID questionnaires, in which the association attenuated to non-significance after correction for HbA1c. We cannot explain the different and contradictory outcome after correcting for HbA1c in the associations with the SF12. However, since these are the only significant outcomes for the health-specific questionnaires and no consistent significant results were found at all for the other regression analyses, these associations are considered chance findings.

Measurement of glycemic variability is complicated. More than 20 criteria to measure glycemic variability are available and one standardized parameter used in literature is lacking[22]. A priori we specified SD as the parameter of choice to describe glycemic variability, since our previous study showed that SD is highly correlated to other parameters of variability[23, 24] and our choice for SD is supported by literature[22, 25]. We did not investigate associations of quality of life with all other different parameters of glycemic variability to prevent multiple testing[26].

The most important limitation of the study is its relative low percentage of patients consenting to the continuous glucose monitoring. Although 503 patients were included in this nationwide long-term biobank initiative study, only 25% consented to also wearing a continuous glucose monitoring. This separate consent could have generated a selection bias. However, from the 126 patients which were included in this sub-study 98% (124) completed the sub-study and could be used for this analysis. Although patients were not told the hypothesis of the study, it remains possible that patients who expected high variability or had a worse quality of life participated in the study more often. This was indeed partly reflected by a slightly lower quality of life in the included group of patients wearing continuous glucose monitoring. Such selection bias could in theory overestimate the associations. However, we adjusted for such selection bias using inverse probability weighting and this did not change our results. We therefore think it did not largely influence our results. Finally, because of the cross-sectional design of this study we can only speculate on the causative effect of glycemic variability on quality of life.

In conclusion, a high glycemic variability was associated with worse quality of life, albeit not independent of HbA1c. Since this association was found in insulin-treated type 2 diabetes patients only, glycemic variability could be a potential treatment goal in this particular group.

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Supplement 1 Linear regression analysis showing association of glycemic variability (SD) with SF12 scores and EQ5D

	Total group		Diet or oral agents only		Insulin treatment	
	β (CI)	**P**	**β (CI)**	**P**	**β (CI)**	**P**
SF12 physical	-1.46 (-3.40-0.49)	0.140	0.86 (-2.84-4.56)	0.643	-2.48 (-5.08-0.12)	0.061
MV	-0.53 (-2.58-1.52)	0.611	1.88 (-2.19-5.95)	0.357	-2.16 (-4.82-0.50)	0.110
MV + HbA1c	-1.17 (-3.56-1.21)	0.334	1.39 (-3.29-6.08)	0.552	-3.08 (-6.07- -0.09)	0.044
SF12 mental	0.31 (-1.45-2.07)	0.727	1.32 (-2.11-4.74)	0.445	-0.12 (-2.46-2.21)	0.917
MV	0.42 (-1.36-2.19)	0.644	0.21 (-3.27-3.68)	0.906	0.72 (-1.59-3.04)	0.534
MV + HbA1c	1.86 (-0.15-3.86)	0.069	3.52 (0.08-6.96)	0.045	1.14 (-1.50-3.78)	0.390
EQ5D VAS	-1.00 (-4.32-2.33)	0.555	1.66 (-4.57-7.88)	0.595	-1.20 (-5.67-3.37)	0.603
MV	-0.65 (-4.15-2.86)	0.715	-0.25 (-6.98-6.48)	0.941	-0.10 (-4.83-4.63)	0.967
MV + HbA1c	-0.20 (-4.31-3.91)	0.925	2.20 (-5.43-9.81)	0.565	-0.81 (-6.21-4.58)	0.764
EQ5D index value	-0.01 (-0.10-0.07)	0.770	0.07 (-0.08-0.21)	0.389	0.00 (-0.12-0.12)	0.970
MV	0.02 (-0.07-0.11)	0.662	0.08 (-0.08-0.25)	0.332	0.04 (-0.08-0.16)	0.490
MV + HbA1c	0.03 (-0.08-0.13)	0.603	0.09 (-0.10-0.28)	0.351	0.04 (-0.10-0.17)	0.601

SF12 physical = SF12 physical component score; SF12 mental = SF12 mental component score; EQ5D VAS = EQ5D visual analogue scale; MV = adjusted for pre-defined covariates (age, sex, duration of diabetes, use of antidepressant drugs and education level); MV + HbA1c = also adjusted for HbA1c

Chapter 4

Real-Time Continuous Glucose Monitoring System for Treatment of Diabetes, a Systematic Review

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* Both authors contributed equally to this review

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Abstract

Aims

This study reviews the effect of real-time continuous glucose monitoring systems in diabetes management.

Methods

A systematic search was performed in PubMed/ MEDLINE and EMBASE for randomized controlled trials comparing real-time continuous glucose monitoring systems with self-monitoring blood glucose or non-real-time continuous glucose monitoring systems.

Results

Nine randomized controlled trials were identified. Two studies used a device which is not on the market anymore. In this review we focus on the other seven studies. Performing a meta-analysis was not possible because of extensive clinical heterogeneity. Six of seven studies showed some positive effect of real-time continuous glucose monitoring systems on HbA1c (HbA1c decrease 0.3-0.7% or 3-8 mmol/mol). In some studies, this effect only was shown in subgroups (compliant adult patients). However, the size of effect may be underestimated by better-than-average results in the control group, as self-monitoring blood glucose measurements are carried out more frequently than in usual clinical practice. Despite the goal of lowering HbA1c, no more severe hypoglycemic episodes were seen, except in one study. In contrast, no positive effect was shown with the real-time continuous glucose monitoring system on hypoglycemia, but randomized controlled trials were not designed or powered to investigate this issue. Time in different glucose strata was assessed only in some trials: two of them showed a significant but small increase in time in euglycemia.

Conclusions

Current evidence shows that the real-time continuous glucose monitoring system has a beneficial effect on glycemic control in adult diabetes patients, without an increase in the incidence of hypoglycemia. Studies in well selected patient groups (pregnancy, history of severe hypoglycemia, type 2 diabetes) are lacking.

Introduction

Optimal glycemic control reduces risk of chronic organ complications in patients with type 1 or type 2 diabetes[1, 2]. The Diabetes Control and Complications trial has shown that achieving good control greatly increases the risk of hypoglycemia. In practice, hypoglycemia forms a major limiting factor despite the best efforts of patients and clinicians. In theory, self-monitoring of blood glucose levels coupled with intensive and extensive ongoing education could help to reduce hypoglycemia. However, the snapshot nature of self-monitoring of blood glucose and the limited number of self-monitoring of blood glucose that are carried out during a day restrict the influence of self-monitoring of blood glucose. The number of self-monitored blood glucose measurements has been shown to correlate with glycemic control[3]; but with four self-monitored blood glucose measurements a day, limited information is available on pre-prandial, postprandial and overnight values[3]**.** In addition, the moment of self-monitoring of blood glucose is chosen by the patient and that moment may not always provide the most optimal and useful information.

The continuous glucose monitoring system is a novel technology potentially revolutionising diabetes treatment by offering longer-term ongoing display of glucose levels. The first continuous glucose monitoring system offered only "off-line" interpretation of the glucose profiles after disconnecting the sensor and uploading the results. In the past years, "on-line" or "real-time" continuous glucose monitoring systems have become available, allowing direct feedback of glucose levels and direct intervention. In theory, the real-time continuous glucose monitoring system would provide a good method to improve glycemic control without the traditional degree of excess hypoglycemia. The continuous glucose monitoring system essentially comprises a needle (containing a glucose-dependent enzyme generating glucose-dependent electrical currents) which has to be inserted into subcutaneous fat, a transmitter connected to the needle (translating and relaying data by infra red technology) and a separate receiver that displays the glucose profile. Calibrating the continuous glucose monitoring system with a number of self-monitoring of blood glucose measurements is necessary. With real-time continuous glucose monitoring systems, glucose thresholds can be set with an alarm going off with glucose levels outside the target area and thresholds can also be set using rates of change.

The real-time continuous glucose monitoring system generates an avalanche of data, but the question of clinical benefit, indications and clinical requirements for implementation have not yet been answered conclusively. Therefore, we conducted a systematic review of all available randomized controlled trials to estimate the effects of real-time continuous glucose monitoring systems on diabetes management.

Patients and Methods

We performed a systematic search for all published randomized controlled clinical trials or meta-analysis/ systematic reviews comparing real-time continuous glucose monitoring systems to self-monitoring of blood glucose and/or the off-line continuous glucose monitoring systems. We searched PubMed/ MEDLINE and EMBASE from 1 January 2005 until 1 January 2010. We restricted the search from 2005 onwards as the use of the real-time devices had not relevantly started before this period. A search including the term "real time" was not comprehensive and important articles were not identified. We therefore extended our search terms. This search strategy for the bibliographic databases combined the following terms (with their synonyms and derivatives) in title/ abstract: "CGMS, monitoring, sensor, continuous, diabetes" [*Appendix 1*]. In addition, we limited the review to English-language articles. In this search, two independent reviewers (LBEAH and WLG) screened the articles, using title/ abstract or full text if necessary, and reviewed reference lists of included articles.

Inclusion and exclusion criteria

Studies included in this review had to be randomized parallel-arm, controlled trials in which the real-time continuous glucose monitoring system was compared with self-monitoring of blood glucose (whether or not in combination with the offline continuous glucose monitoring system). Studies included children and adults, as well as type 1 and type 2 diabetes and all kind of devices for real-time continuous glucose monitoring (this included devices with current use as well as devices that had already been withdrawn from the market at the time of this review). Withdrawal from the market was not an exclusion criterion in our search, as this could potentially lead to selection bias with exclusion of negative studies as negative studies may be more likely to be associated with these devices.
Reasons for exclusions were studies on post-pancreatic/islet cell transplant patients and studies with settings like Intensive Care, Cardiac Monitoring Unit, pre- and post-operation and studies with a follow-up of less than 6 weeks as it takes minimal 6 weeks to detect a meaningful change in HbA1c. In case of a duplicate publication, the publication with the most comprehensive information was used.

Outcomes of interest

The primary outcome was improvement in diabetes control according to an absolute reduction in HbA1c in a head-to-head comparison or a comparison of absolute change form baseline between both groups. The secondary outcomes were: severe hypoglycemic episodes (as defined by the investigators), time spent in different glucose strata (hypoglycemic, euglycemic, hyperglycemic), local adverse effects, quality of life and compliance.

Data collection

Relevant data were extracted on predesigned forms. These forms included information about author, publication year, country, duration of the trial, number of patients in the study and characteristics of these patients (type of diabetes, age, duration of diabetes, therapy, HbA1c at start of study, frequency of self-monitoring of blood glucose measurements) and information on type of device, type of usage (intermittent/ continuous) and duration of usage.

Statistics

We could not perform a meta-analysis because of extensive clinical heterogeneity on many aspects such as design, type of diabetes (mostly type 1 diabetes, but also type 2 diabetes, or mixed), age of participants (children, adolescents, adults), therapy (multiple daily injections of insulin or continuous subcutaneous insulin infusion) and glycemic control.

Quality control

The methodological quality of the studies that met the inclusion criteria was assessed using the components of the study design most closely aligned to internal validity, as proposed by the Dutch Cochrane Centre[4]. These components include: adequate description of randomization, blinding of patients and outcome assessors, and adequate description of follow-up and withdrawals. The higher the Cochrane score, the higher the methodological quality of the study. A score ≥ 4 was defined as of sufficient quality for this review. In case of doubt, consensus was reached in an open discussion with the third author (HWdV).

Results

Literature search and study selection

The search strategy resulted in 1018 articles in PubMed/ MEDLINE and 223 in EMBASE. After screening for inclusion and exclusion criteria, 18 articles seemed relevant. Of these remaining 18 articles, nine were excluded after reading full paper: eight turned out to be non-randomized controlled trials and one was too short of duration (*Figure 1*). The quality assessment of the remaining nine articles[5-13] showed that all articles had a Cochrane score ≥ 4 and were therefore included in this review [*Appendix 2*].

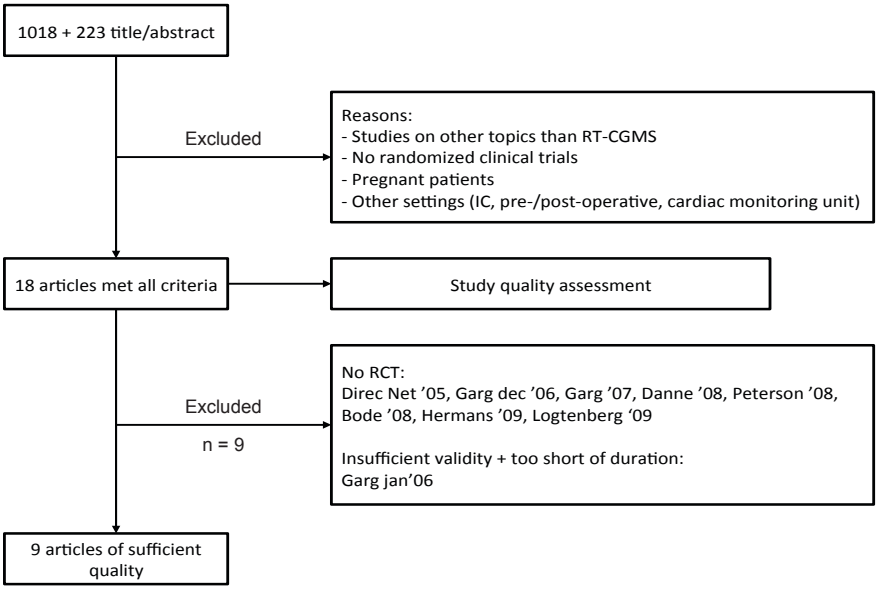

Figure 1. Scheme of included and excluded studies.
IC = intensive care; RCT = randomized controlled trial; RT-CGM = real-time continuous glucose monitoring system

Description of studies and patient characteristics

A description of design, characteristics and outcomes of studies included in the systematic review are presented in *Tables 1 and 2*. Two studies were in fact reports of two separate subpopulations (patients with an HbA1c > 7% (> 53 mmol/mol) and patients with an HbA1c < 7%) (<53 mmol/mol) from one larger intervention study[5,12]; Juvenile Diabetes Research Foundation Studies 1 and 2). Study duration ranged from 12 weeks to 18 months. All studies had parallel study design[5-12]; however, in one study some variables were only investigated in a single arm constructure[13]. Studies included patients with either type 1 or type 2 diabetes or both, different age groups, different insulin treatment regimens and different degrees of glycemic control as expressed as HbA1c (ranging from poor to excellent). Four of seven studies used self-monitoring of blood glucose and blinded continuous glucose monitoring data as control. Two of seven studies used self-monitoring of blood glucose data as control.

Studies using Glucowatch (GW2B) device

Although "today-use" was not a inclusion criterion, we will only describe seven of the studies; the two studies that used[6,7] a real-time continuous glucose monitoring system device (GW2B) that is not on the market anymore were described separately. This device caused a lot of skin irritations (100% in DirecNet group[6] and 49% in Cooke *et al*[7]), which led to very low compliance rates and many users stopped early (27 and 80% of users, respectively). These were both negative studies, but we consider these results not to be representative for the effect of real-time continuous glucose monitoring systems in general because, when using this device, compliance is of great importance. Therefore, in the following section of the Results we will only describe the other seven studies.

HbA1c

HbA1c was reduced to a greater extent in the real-time continuous glucose monitoring system group than in the control group in three studies[8, 10, 13]. The study by Tamborlane *et al.* also showed a significant difference, but only in the subgroup of patients > 25 years[12]. In another study in very adequate controlled patients (baseline HbA1c 6.5%, 48 mmol/mol), the real-time continuous glucose monitoring system did not show a decrease in HbA1c with that system, but there was an increase in HbA1c in the control group[5]. Raccah *et al.*[11] showed only significant differences in the compliant patient group, but when the complete patient population was analysed only borderline significant results remained. The only study not showing any difference between the real-time continuous glucose monitoring system and the control group was the study by Hirsch *et al*.[9] in poorly-controlled patients with continuous subcutaneous insulin infusion. The study of O'Connell *et al*.[10] was the only study with a head-to-head comparison of HbA1c as primary variable at the end of the intervention showing a statistically significant improvement in HbA1c with the real-time continuous glucose monitoring system. In conclusion, six studies showed some positive effect (0.3-0.7% or 3 -8 mmol/mol) of the real-time continuous glucose monitoring system on HbA1c compared with the control.

Symptomatic hypoglycemia

None of the seven studies demonstrated a positive effect of the real-time continuous glucose monitoring system on the incidence of severe hypoglycemia. One study[9] actually showed an increase in severe hypoglycemia. Two studies showed a decrease in HbA1c, in absence of severe or non-severe hypoglycemia in the real-time continuous glucose monitoring system group[5, 12]. This combined end-point is of clinical interest as the lowering of HbA1c without increase in hypoglycemia is an ultimate goal in diabetic management.

Table 1. Characteristics of included trials

Study	Basics		Inclusion			
	Duration of trial	Sample size (SG/ CG)	Type diabetes	Age inclusion (mean SG vs CG)	Duration diabetes (mean SG vs CG)	Diabetes treatment at baseline (SG vs CG)
Beck et a.l[5] (JDRF) 2009 United States	6 months	67/62	T1DM	≥8 yr (29 vs 32 yr)	≥1 year (5-25 vs 4-28 yr)	CSII or MDI (93% vs 79% CSII 7% vs 21% MDI)
Deis et al.[8] 2006 Sweden	3 months	total 156	TIDM	≥8 yr (unknown)	Unknown	CSII or MDI (unknown)
Hirsch et al.[9] 2008 United States	6 months	66/72	T1DM	12-72 yr (33 vs 33 yr)	> 1 year (21 vs 17 yr)	≥6 months CSII (unknown)
O'Connell et al.[10] 2008 Australia	3 months	31/31	T1DM	13-40 yr (23 vs 23 yr)	> 1 yr (11 vs 9 yr)	CSII > 3 months (2.4 vs 1.9 yr)
Raccah et al.[11] 2009 France	6 months	55/60	T1DM	2-65 yr (28 yr)	≥ 1 year	MDI → CSII during the study
Tamborlane et al.[12] (JDRF)2008 United States	6 months	165/157 ±100 per group (group 1: 8-14 yr group 2: 15-24 yr group 3: ≥25 yr)	T1DM	≥ 8 yr (G1:11 yr G2:18 yr G3:42 yr)	> 1 year (G1:6 yr G2: 9 yr G3:22yr)	CSII or MDI (G1: 85%CSII vs 15% MDI G2: 70 vs 30% G3: 85%vs 15%)
Yoo et al.[13] 2008 Korea	3 months	32/33	T2DM	20-80 yr (55 vs 58)	> 1 year (12 vs 13 yr)	Tablets,insulin,both (45%vs36% tablets 14%vs18% insulin, 38%vs43% both)
Cooke et al.[7] 2009 United Kingdom	18 months	100 RT-SG 102 blind-SG 100 extra care CG 100 standard CG	57%T1DM 41%T2DM	≥ 18 yr (52 yr)	> 6 months (16 yr)	97% MDI 2% CSII
DirecNet study group[6] 2005 United States	6 months	99/101	TIDM	7-18 (12.3 vs 12.7 yr)	≥ 1 year (5.3 vs 5.4 yr)	≥ 1 year CSII (46 vs 47%) or MDI (54 vs 53%)

*All trials have a randomized, parallel-group design.
GW2B = GlucoWatch G2 Biographer; CG = control group; CGMS = continuous glucose monitoring system;
CSII = continuous subcutaneous insulin infusion; MDI = multiple daily injections; JDRF = Juvenile Diabetes Research Foundation;
Min. = minimum; RT = real-time; SG = sensor group (real-time continuous glucose monitoring system); T1DM = Type 1 diabetes;
T2DM = Type 2 diabetes.
DexCom, San Diego,California; Medtronic, Minneapolis; Abbott, North Chicago, Illinois; Cygnus, Michigan area

Inclusion	Methods		
Baseline HbA1c inclusion (mean SG vs CG)	**Frequency of CGMS use in intervention group (total number)**	**Methods of control measurements**	**Type CGMS**
≤7.0%, 53 mmol/mol (6.4 vs 6.5%, 46 vs 48 mmol/mol)	Daily; at least 70%	SMBG ≥3x/day Blind CGMS twice	DexCom Seven (Dexcom) Minimed Paradigm (Medtronic) FreeStyle Navigator (Abbott)
≥8.1%, 65 mmol/mol (unknown)	two groups: SG1. 3 months continue SG2. biweekly 3-days	Unknown	Guardian RT (Medtronic)
≥ 7.5%, 58 mmol/mol (8.5 vs 8,4%, 69 vs 68 mmol/mol)	daily	SMBG unknown 2x 3 days blind CGMS	Paradigm 722 (Medtronic)
≤ 8.5%, 69 mmol/mol (7.3%, 56 mmol/mol vs 7.5%, 58 mmol/mol)	Continuously	SMBG Min 4x/day 2x 6 days blind CGMS at baseline and end	MiniMed Paradigm (Medtronic)
≥ 8.0%, 64 mmol/mol (9.1/9.3%,76-78 mmol/mol)	Min 70%	SMBG Min 3x/day	MiniMed Paradigm (Medtronic)
7-10%, 53-86 mmol/mol G1: 7.9%,63 mmol/mol G2: 7.9%, 63 mmol/mol G3: 7.6%, 60 mmol/mol)	daily	SMBG Min 4x/day + 2x blind CGMS (wk 13+26)	DexCom Seven(Dexcom) MiniMed Paradigm (Medtronic) Freestyle Navigator (Abbott)
8-10%, 64-86 mmol/mol (9.1%, 76 mmol/mol vs 8.7%, 72 mmol/mol)	1x/month for 3 days	SMBG Min 4x/wk (fasting + postprandial)	Guardian RT (Medtronic)
≥ 7.5%, 58 mmol/mol (9.1%, 76 mmol/mol)	0-3 months: > 4x/month and < 4x/week 3-18 months: "as often as desired"	0-3 months: SMBG daily + blind CGMS 72h at 0, 6, 12 weeks 3-18 months: SMBG daily + Blind CGMS once a month	GW2B (Cygnus)
7-11%, 53-97 mmol/mol (8.0%, 64 mmol/mol)	"As much as possible"	Min 4x/day + 2x blind CGMS (begin and end of study)	GW2B (Cygnus)

Table 2. Results

Study	HbA1c	Severe hypoglycemia	Time in hypoglycemia	Time in range
Beck et al [5] (JDRF) 2009	SG: 6.4 → 6.4 % (46→46 mmol/mol) CG: 6.5 → 6.8 % (48→51 mmol/mol) (p<0.001)	NS	SG: 91→45 min CG: 96→91 min (borderline sign)	SG: 1063→1063 min CG: 972→949 min? (p=0.003)
Deiss et al.[8] 2006	SG1: 9.5→8.5 % (80→69 mmol/mol) SG2:9.6→8.9 % (81→74 mmol/mol) CG: 9.7→9.3 % (83→78 mmol/mol) (p=0.003 for continuous use (SG1) NS for biweekly use (SG2))	NS	Not measured	Not measured
Hirsch et al.[9] 2008	NS	SG: 11 events CG: 4 events (p=0.04)	SG: stable CG: increase (p=0.0002)	Not measured
O'Connell et al.[10] 2008	SG: 7.3 → 7.1% (56→54 mmol/mol) CG: 7.5 → 7.8% (58→62 mmol/mol) (p=0.009)	None	NS	NS
Raccah et al.[11] 2009	NS full analysis set (p=0.087) In per protocol set: SG: 9.2→8.2% (77→66 mmol/mol) CG: 9.3→8.8% (78→73 mmol/mol) (p=0.004)	NS	NS	Not measured
Tamborlane et al.[12] (JDRF) 2008	In adults: SG:7.6 → 7.1% (60→54 mmol/mol) CG:7.6→7.6% (60→60 mmol/mol) (p<0.001) Children and adolescents NS	NS	NS	SG: 854→986 CG:811→840(min/day) (P<0.001) Children and adolescents NS
Yoo et al.[13] 2008	SG: 9.1 → 8.0% (76→64 mmol/mol) CG: 8.7 → 8.3% (72→67 mmol/mol) (p<0.001)	NS	Not measured between groups NS within the SG	Not measured between groups NS within the SG
Cooke et al.[7] 2009	Less HbA1c improvement in the SG vs CG Actual data not given (p=0.02)	Not measured	Risk reduction for clinical hypoglycemia of RR 0.83 (0.67-0.98) for standard control versus SG	Not measured
DirecNet study group[6] 2005	NS	NS	Not measured	Not measured

→ = baseline to end of study

CG = control group; CGMS = continuous glucose monitoring system; JDRF = Juvenile Diabetes Research Foundation; MAGE = mean amplitude of glycemic excursions; NS = not significant; RR = relative risk; RT = real-time; SG = sensor group (RT-CGM)

Time in Hyperglycemia	Adverse effects	Compliance/ miscellaneous
NS	NS	Compliance was very good Decrease in HbA1c without increase in hypoglycemia was higher in the SG (p<0.001)
Not measured	Unknown	Compliance unknown
NS	1x skin abscess	Compliance very good Compliance was related to reduction in HbA1c
NS	Only mechanical problems	Overall compliance unknown Lower hbA1c in patients with > 70% compliance Effect on variability NS
SG: Δ=-3.5 (h/day) CG: Δ =-0.7 (h/day) (p<0.005)	NS	Compliance: 75% in adults, 68% in children, 52% in adolescents Variability decreased in the sensor group
SG: 497→394 CG: 549→519 (min/day) (p=0.002) Children and adolescents NS	Very infrequent	Compliance (>6 days/ week): 83% in adults, 50% in children, 30% in adolescents HbA1c < 7.0 without severe hypoglycemia was higher in the SG in adults and children
Not measuredbetween groups Significant reduction within the SG	0% skin-reactions	Compliance unknown Better exercise time+ calorie intake, MAGE, BMI+weight in SG compared to baseline (not compared to CG)
Not measured	Skin reactions 49%, difficulties in usage (10%)	Compliance was bad (80% stopped wearing the device) Cost analysis not significant
Not measured	100% skin irritation	compliance was bad (27% stopped, none used the sensor > 3 times a week)

In general, no decrease, but also no increase in hypoglycemia, was observed.

Time in predefined glucose strata

One study uses "time-in-target-glycemia" as the primary endpoint[10]. During the study, no change in the intervention or control group and no difference between the groups was observed. Tamborlane *et al.* showed a decreased time in hyperglycemia and increased time in target in adults compared with the control group. Another study showed a decrease in euglycemia in the control group, but not in the real-time continuous glucose monitoring system group[5]. The study of Raccah *et al*.[11] only showed a decrease in time in hyperglycemia, without any effect on time in euglycemia. Considering time in hypoglycemia, two studies showed some improvement. One study only showed an increase in the control group without a change in the sensor group[9], the other showed a borderline significant decrease in time spent in hypoglycemia in the real-time continuous glucose monitoring system group[5]. The study by Yoo *et al.*[13] showed that, within the real-time continuous glucose monitoring system group, time in hyperglycemia was decreased as compared with baseline, but data of the control group are lacking.
So, some evidence exists that the distribution of glucose values over the various strata can be improved using the real-time continuous glucose monitoring system, although the minority of this analysis was significant.

Adverse events

The device was well tolerated in all seven studies. Adverse events were infrequent and not significantly different from the control group. Adverse events consisted mainly of skin irritation.

Compliance

Compliance with sensor use was relatively good in all seven studies using different devices, but fell over time. Three trials showed increased HbA1c improvement in patients with better compliance[9-11]; one study showed that, adjusted for baseline values, HbA1c was 0.51% lower in participants who wore the sensor ≥70% of the total study period (98%CI 0.04-0.98%, p=0.04)[10]. Another study showed that each 10% increase of time the sensor was used was associated with a 41% increase in the probability of a 0.5% reduction in HbA1c[9]. The last study only showed a significant difference in HbA1c in fully compliant patients, whereas in the whole group significance was only borderline[11]. Compliance was dependent on age group; it was highest in adults, lower in children and the lowest in adolescents[11,12].
In all, compliance was reasonable and an important factor for the effect of the real-time devices.

Other considerations

Costs were only analyzed in one study; Cooke *et al.*[7] showed costs did not differ significantly between treatment and control group.
Quality of life was not assessed in any of the studies.
Three studies investigated effects of real-time continuous glucose monitoring systems on glycemic variability[10, 11, 13]. Two of them showed a significant reduction of variability in patients using the device[11, 13]. Although, in the study of Yoo *et al.*[13], this was a within group effect and a head-to-head comparison between the sensor group and the control group was lacking. The other studies did not investigate this endpoint.

Discussion

This systematic review of nine randomized controlled trials, in which we focus on the seven trials about currently available devices, published in the last 5 years indicates that the real-time continuous glucose monitoring system has a considerable potential to be an effective tool for improving glycemic control in adults with type 1 diabetes. Less convincing evidence is available for children and type 2 diabetes.
The diversity of study design and populations, the lack of studies in subjects with specific clinical demands such as recurrent severe hypoglycemia or pregnancy, as well as the complex nature of the intervention itself, preclude simple translation to clinical practice. A number of specific issues has to be addressed before more widespread implementation can be wholeheartedly supported.
Firstly, the choice of the optimal or most relevant variable of glycemic control to assess the effect of intervention with the real-time continuous glucose monitoring system is a major issue. In most studies, HbA1c was taken as the principal variable of glycemic control and the primary end point. In only one study, a head-to-head comparison at the end of the study was performed; in all other studies, the change in HbA1c between start and the end of study was compared between intervention and control groups. In general, HbA1c decreased irrespective of the baseline HbA1c, indicating that there is no reason to exclude patients on the basis of baseline HbA1c, except those with a very high HbA1c in whom other issues regarding treatment and self-management require attending to first.
However, HbA1c does not reflect the complexities of glycemic control and patients can display wildly and widely swinging glucose levels (high glucose variability), or severe hypoglycemic and hyperglycemic episodes, with nevertheless a reasonable HbA1c-value. The question then is how to attain improvement of glycemic control in such patients. Glucose variability, for example by the simple calculation of the standard deviation, could serve as the endpoint in subjects with a wide range of

glucose values[14]. Glucose variability was shown to decrease in two of the three studies that used this as an endpoint[11, 13].

As an alternative approach, one could use the variable of "time spent in preset glucose ranges". Improvement is than defined as decreased time spent in hyperglycemia or time spent in hypoglycemia and/or increased time spent in euglycemia. Comparing the four studies employing this variable, however, shows that there is no consensus on the definition of euglycemia, hypoglycemia and hyperglycemia. Lower limits of euglycemia ranged from 2.8- 4.4 mmol/l, upper limits from 10.0-13.9 mmol/l.

In summary, the best variable to assess glycemic control and the complexities of control remains a major unsolved issue in the assessment of the true value of the real-time continuous glucose monitoring system.

Secondly, apart from a general effect on glycemic control, some subjects will present with a specific clinical condition, most notably recurrent severe hypoglycemia and hypoglycemia unawareness. The reviewed studies could not give a clear-cut answer to the question of whether the real-time continuous glucose monitoring system could be helpful in these patients. In some studies, patients with a history of severe hypoglycemia were specifically excluded[10] and in other studies episodes of severe hypoglycemia were not endpoints and were not reported[7]. In the Juvenile Diabetes Research Foundation studies[5, 12], no difference in the incidence of severe hypoglycemia was found, but the percentage of patients with severe hypoglycemia at baseline was not mentioned. The same was true for the study of Raccah *et al.*[11] and the much smaller studies by Deiss *et al.*[8], Yoo *et al.*[13] and Hirsch *et al.*[9]. In summary, no effect of the real-time continuous glucose monitoring system seems apparent on the incidence of severe hypoglycemia in patients not specifically selected for that problem. This does not at all exclude a beneficial effect on patients with frequent severe hypoglycemia. Although common clinical sense may suggest trying the real-time continuous glucose monitoring system in patients so afflicted, the evidence of solid randomized trial to support this line of action is lacking.

Thirdly, compliance is a major issue and some studies clearly reported a positive association between the degree of compliance and the effect on the primary end point[9-11]. Compliance decreased during the course of the trial, which could be linked to sensor-related skin problems, waning of the initial enthusiasm for the active intervention, or to the burden of the intervention itself (the constant feedback of data and/or the frequent alarms, especially during the night). As said before, compliance with the Glucowatch was especially poor as almost all patient suffered form severe skin reactions induced by the sensor. We described the studies using this device separately because, even if the sensor itself would had been excellent, this device would have been unsuitable for normal practice. Apart from this specific device, no major adverse events were reported; safety is thus not an issue in these devices.

Fourthly, the trial design and the comparator groups deserve attention. The nature of the intervention precludes blinding of the subjects and the study personnel during the trial. Endpoints related to sensor data (like "time spent in hyperglycemia") can be analysed blindly offline, but "conclusions about clinical events (severe hypoglycaemia)" has to be adjudicated by an independent committee to prevent bias. In randomized controlled trials, it could be that the control subjects could perform better than they normally would because they participate in a trial. The frequency of self-measurement of glucose levels required in the studies is higher than in normal daily life and the study control group may therefore not be a realistic reflection of the average diabetes patient. Thus, the effect of the real-time continuous glucose monitoring system might be underestimated.

Not only the frequency of self-measurement of blood glucose levels is important, but also the general counselling and support of the patient must be comparable in both the intervention and the control group and clearly stated in the description of the study.

Finally, the real-time continuous glucose monitoring system requires an extensive and detailed care and counselling system and adequate training of the healthcare professionals. Some studies[9-11] only provided patients with the device without proper training, sometimes because the researchers themselves did not have enough experiences, as they admitted in their conclusion[9]. But assessing the effect on HbA1c with such a new and precise device, a more proactive approach is needed to inform the user how to react on static and dynamic alerts. Without such a collaborative effort, the effect the real-time continuous glucose monitoring system may be underestimated. This means that the real-time continuous glucose monitoring system requires a substantial professional investment and a major contribution from the patient. These factors may be seen as prerequisite for the implementation of this novel and expensive technique and may limit the number of centres offering the real-time continuous glucose monitoring system.

In conclusion, current evidence shows that the real-time continuous glucose monitoring system has a general beneficial effect on glycemic control, but that information on specific clinical indications such as recurrent severe hypoglycemia, type 2 diabetes or pregnancy is lacking. The technique requires a long-term commitment of the health care professionals and the patient to translate the potential effect into common practice. Also, more information is needed on longer-term outcomes, including compliance and quality of life.

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Appendix I

Search:

Pubmed
CGM[Title/Abstract] OR CGMS[Title/Abstract] OR "continuous glucose monitoring"[Title/Abstract] OR "continuous glucose"[Title/Abstract] OR "glucose monitoring"[Title/Abstract])
OR observing[Title/Abstract] OR observed[Title/Abstract] OR observing[Title/Abstract] OR monitoring[Title/Abstract] OR monitored[Title/Abstract] OR monitors[Title/Abstract])
AND (continuous[Title/Abstract] OR continuing[Title/Abstract] OR persisting[Title/Abstract] OR continue[Title/Abstract])
OR Sensor[Title/Abstract] OR glucose sensor[Title/Abstract] OR sensing[Title/Abstract] OR sensoring[Title/Abstract]) AND continuous[Title/Abstract] OR continuing[Title/Abstract] OR persisting[Title/Abstract] OR continue[Title/Abstract])
AND
(diabetes[Title/Abstract] OR diabetes mellitus[Title/Abstract])
AND
"2005/01/01"[EDAT] : "2009/08/01"[EDAT]
AND
English[lang]

Embase
"Continuous glucose monitoring system"
2005-2010

Appendix II

Study	Randomized	Blind randomized	Blind analysis	Comparable groups	Loss to Follow-up	Intention to treat	Equal therapy	Results correctly shown	Score
Beck[5]	yes	?	no	yes	2/129	yes	yes	yes	6
Cooke[7]	yes	yes	no	yes	74/404	no	yes	yes	5
Deiss[8]	yes	?	?	?	5/162	yes	yes	?	≥4
DirecNet[6]	yes	no	yes	yes	1/200	yes	yes	yes	6
Hirsch[9]	yes	?	no	yes	8/176	no	yes	yes	5
O'Connell[10]	yes	yes	no	yes	7/62	no	yes	yes	6
Raccah[11]	yes	?	yes	yes	13/128	yes	yes	yes	7
Tamborlane[12]	yes	?	no	yes	5/322	yes	yes	yes	6
Yoo[13]	yes	yes	?	yes	8/65	?	yes	yes	6

Chapter 5

Patients with Type 2 Diabetes Mellitus Failing on Oral Agents and Starting Once Daily Insulin Regimen; a Small Randomized Study Investigating Efffects of Adding Vildagliptin

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Abstract

Background

The addition of a DDP4-inhibitor to existing insulin therapy reduces HbA1c. However, no data exist about the addition of these agents at the beginning of insulin treatment in type 2 diabetes while this could especially be interesting because it is during this period that considerable residual beta cell function is still present. The benefit of such a strategy could be a lower insulin dose required for glycemic control. The hypothesis of our study was that adding a DPP4-inhibitor at the beginning of insulin treatment could lead to less exogenous insulin requirement, a reduction of hyperinsulinemia and side effects (hypoglycemia and weight gain), less glucose variability and improvement of insulin and glucagon dynamics during a mixed meal test.

Results

In this small clinical trial (trial registration NTR2022) 9 patients were randomized to receive vildagliptin and 6 to receive placebo in addition to start of once daily insulin treatment. Unfortunately, due to a difficult inclusion, the preset sample size of 40 patients could not be met. Median units of insulin at the end of the study was 47 U in the placebo group and 34 U in the vildagliptin group. Median glycemic variability (SD) at the end of study was 2.1 in the placebo group and 1.5 in the vildagliptin group. Median weight gain at the end of study was 3 kg in the placebo and 0.5 kg in the vildagliptin group. Occurrence of hypoglycemia was low in both groups. Insulin, C-peptide, glucose and glucagon levels were comparable during mixed meal tests

Conclusions

This small randomized study did not have sufficient power to detect effects of the addition of vildagliptin to the start of once daily long-acting insulin. However in our opinion adding a DPP4-inhibitor, especially in this group remains a very interesting approach. This study could be used as a guidance for larger studies that are required to investigate the effects of this intervention on insulin requirements, glycemic variability, hypoglycemia and weight gain.

Background

Due to the progressive nature of the disease, most patients with type 2 diabetes ultimately fail on oral glucose-lowering drugs and therefore require insulin therapy[1, 2]. Often considerable doses of insulin are needed and weight gain and hypoglycemia can occur[1]. The dipeptidyl peptidase-4 inhibitor (DPP4-inhibitor) vildagliptin is an oral glucose-lowering drug that leads to glucose-dependent insulin secretion and improvement of alpha cell function[3]. A reduction in glycated haemoglobin (HbA1c) and less hypoglycemia was shown in a previous study adding vildagliptin on top off an existing insulin regimen[4]. Multiple randomized-controlled trials investigating the effect of DPP4-inhibitor added to existing insulin have been performed since[4-14]; however, no data exist about vildagliptin use at the start of insulin treatment in patients with Type 2 diabetes. Adding a DPP4-inhibitor at the beginning of insulin treatment could especially be interesting because it is during this period that considerable residual beta cell function is still present. The benefit of such a strategy could be a lower insulin dose necessary for glycemic control. To study this, we titrated insulin in all patients to the best achievable degree of glycemic control. Therefore, we did not use HbA1c as the primary endpoint, as most previous studies on DPP4-inhibitors and insulin did, but the required insulin dose instead.
The aim of the study was to investigate whether or not the addition of a DPP4-inhibitor to start of insulin treatment could lead to lower exogenous insulin requirements together with lower glucose variability, less weight gain, less hypoglycemia, and improved insulin and glucagon dynamics after a mixed meal test through improvement in alpha and beta cell function.

Methods

Design

This study was set up as a double-blind parallel-arm placebo-controlled randomized monocenter trial (16 weeks) comparing the effects of adding vildagliptin or placebo to the start of insulin in patients with type 2 diabetes. Patients were included from December 2009 – May 2012.

Patients

Patients who were scheduled by their treating physician to start once-daily long-acting insulin were eligible. Inclusion criteria were: Type 2 diabetes, failing on maximally tolerated oral-glucose-lowering medication, BMI 25–35, HbA1c 53–75 mmol/mol (7.0-9.0%) and age 25–75 years. Exclusion criteria were:

pregnant women or women in the fertile period of life without adequate birth-control, type I diabetes, or another type of diabetes (for example pancreatic injury, prednisone induced), acute metabolic diabetes complications during the last 6 months, severe cardiac (left ventricle ejection fraction (LVEF) < 30%) or (a history of) hepatic failure (transaminases > 3 times elevated), or renal impairment (creatinine clearance <50 ml/min).

Randomization

Patients were randomized to receive vildagliptin (50 mg twice daily) or matching placebo (twice daily). To prevent confounding by BMI, patients were randomized after stratification for BMI (using two different randomized lists for BMI 25–30 kg/m2 or BMI 30–35 kg/m2). Consecutive patients were allocated to the two different groups using two computer-generated randomized lists (block size 4), which were stored in sealed envelopes. Randomization and distribution of blinded study medication was performed by a person not related to the study (pharmacy). Patients and care providers were unaware of the randomization code.

Treatment

Besides the study medication, all patients started with once daily long-acting insulin glargine at bedtime in combination with a fixed dose of metformin (twice daily 850 mg). Other glucose lowering drugs were terminated. The combination with metformin was used since this is standardized approach in Dutch clinical practice. The dose of insulin glargine was protocolized and titrated based on daily fasting blood glucose measurements of the patients and an algorithm comparable to the one published by Davies *et al.*[15]. In short, all patients started with 8 units insulin glargine at bedtime and performed daily fasting glucose measurements. The insulin dose was increased based on the mean of the last glucose measurements. This was performed twice a week for the first 3 weeks and once a week during the remainder of the study. The insulin dose was raised as follows: mean glucose 5.5-6.7 mmol/L: raise of 0–2 units, 6.7-7.8 mmol/L: 2 units, 7.8-10 mmol/L: 4 units , >10 mmol/L: 6–8 units increase. The dose was only raised in the absence of hypoglycemia.

Outcomes

Primary outcome: units of insulin at the end of the study.
Secondary outcomes : 1) glycemic variability estimated by the standard deviation (SD) of 48 hours glucose values at the end of the study as measured by three days blinded continuous glucose measurement (CGM))[16] 2) change in weight 3)

hypoglycemia (defined as the total number of hypoglycemia during the study period (any glucose self-measurement < 4.0 mmol/L or symptoms which the patients recognizes as hypoglycemia and clinically judged by the investigator as hypoglycemia)) 4) severe hypoglycemia (defined by help needed from others, seizure, or coma) 5) alpha and beta cell function (between group comparison of glucose, insulin, C-peptide and glucagon levels, measured after standardized meal) 6) cardiovascular analysis; change in blood pressure (mean ambulant 24 hour arterial pressure) and change in plasma lipids 7) change in skin Advanced glycation end products (AGEs) (measured as skin-autofluorescence by the AGE-reader)[17, 18].

Adverse events (AE): any (worsening of an) undesirable sign, symptom, or medical condition that was noted by the patient occurring after starting the study drug even if the event was not considered to be related to the study drug. Serious adverse events (SAE): AE judged as medically significant, requiring in-patient hospitalization, prolongation of existing hospitalization, or resulting in persistent or significant disability or incapacity or are life-threatening or fatal.

Study procedures

Age, BMI, duration of diabetes, medication use, history of diabetes complications, blood pressure, prior hypoglycemia (defined as symptomatic hypoglycemia in the past year), HbA1c, glucose variability and laboratory values were recorded at baseline. Patients were seen every 4 weeks in the out-patient clinic in our academic hospital and with telephone consultation every week. Drug accountability and laboratory measurements were performed at 8 and 16 weeks. Continuous glucose monitoring and ambulant blood pressure measurement were repeated at the end of the study. At the end of the study a standardized mixed meal test (MMT) was performed. Patients arrived fasting at the outpatient clinic and took their medication (metformin and study medication) at t = 0, after blood samples were drawn as a baseline (t = 0). At t = 30 blood samples were drawn again and they consumed a standardized breakfast within 15 minutes (containing 522 Kcal; 27 gr protein, 60 gr carbohydrates, 18 gr fat). Blood samples for glucose, insulin, C-peptide and glucagon were then drawn at t = 45, 60, 90, 120, 150 and 210 minutes. Insulin and C-peptide were measured using an electrochemiluminescence immunoassay on the Modular E170 (Roche Diagnostics GmbH, D-68298 Mannheim, Germany). Finally glucagon was measured with a competitive radioimmunoassay (Glucagon KGND1, Siemens Healthcare Diagnostics Inc, Los Angeles, USA).

The study was conducted using Good Clinical Practice according to the declaration of Helsinki. The protocol was approved by the ethics committee of the UMCUtrecht and all patients provided written informed consent. This

investigator-driven study was designed, performed and analyzed by the researchers of the UMCUtrecht. Novartis provided study medication and a research grant. The protocol of this trial was published before start of the trial at www.trialregister.nl (NTR2022).

Statistics and sample size calculation

Results are presented as median and range, categorical parameters as percentages. Areas under the curve of glucose, insulin, C-peptide and glucagon were calculated using the trapezoidal method. Since we did not meet our intended sample size results are presented in a pure descriptive way.
The group size was based on the primary study parameter: the absolute difference in daily insulin dose of long-acting insulin between the two groups at the end of the trial. A previous study in patients starting on once-daily insulin showed a mean insulin glargine dose of 57 +/− 15 units[19] A meaningful decrease would be a difference of at least 14 units. A similar decrease (25%) in insulin dose has been observed when adding metformin to existing insulin treatment[20]. With a beta of 0.2 and a one-sided alpha of 0.05, the minimum number of patients in each group is 15. To correct for unforeseen circumstances, we planned to include 20 patients in each group.

Results

In this study 19 patients were screened and 4 excluded (reasons for exclusion in *Figure 1*). Nine patients were randomized to receive vildagliptin and 6 patients to placebo (*Figure 1*). This study was designed to include 40 patients in 1 year. Since inclusion rate was much slower than expected, the inclusion period was extended by more than a year. However, the number of 40 patients could still not be met and inclusion was therefore terminated after 15 patients.
Table 1 shows the baseline characteristics, which were comparable in both treatment groups.
Results are summarized in *Table 2*. Median number of units of insulin in the placebo group was 47 and in the vildagliptin group 34. In both groups compliance was high and HbA1c decrease was comparable. Median glycemic variability (SD) in the placebo group was 1.8 at the beginning of the study and 2.1 at the end, in the vildagliptin group glycemic variability was 1.7 and 1.5 respectively. Median weight increase at the end of the study was 3 kg in the placebo and 0.5 kg in the vildagliptin group. Occurrence of hypoglycemia was low and comparable in both groups. Median systolic blood pressure change in the vildagliptin group was + 2.5 and −3 mmHg in the placebo group. LDL and SAF only showed very minor changes.

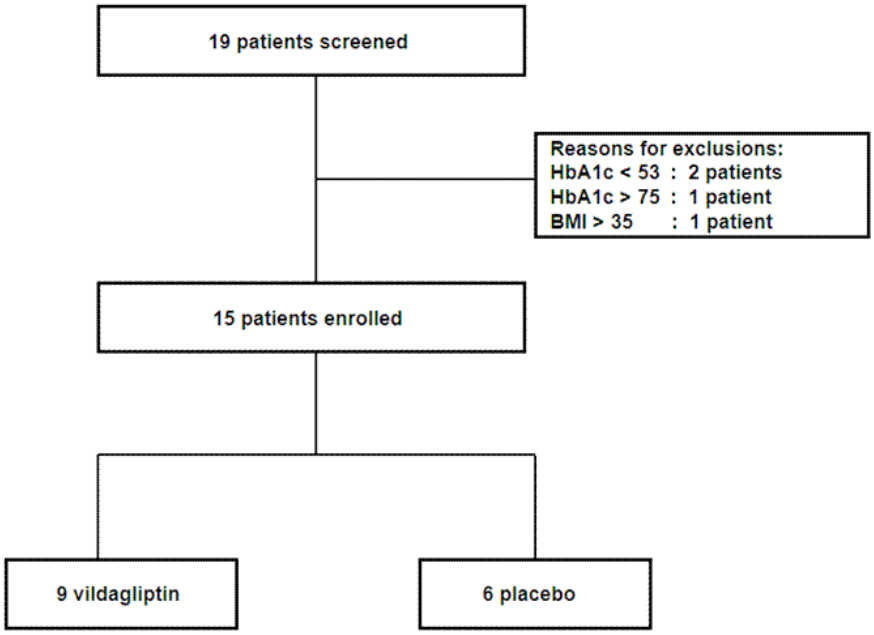

Figure 1 Randomization scheme of the trial

Table 1 Base-line characteristics

	Placebo	Vildagliptin
N	6	9
Age (years)	60 (39–67)	64 (42–67)
BMI (kg/m^2)	32 (26–35)	32 (27–34)
Female (%)	17%	22%
Duration DM (years)	5.5 (1–23)	6.0 (2–15)
Prior medication		
metformin/SU/TZD/other (%)	100/67/0/33%	100/89/11/22%
Diabetes complications		
any microvascular complication (%)	17%	33%
any macrovascular complication (%)	0%	33%
BP (syst/diast in mmHg)	128/78	126/73
HbA1c at start of the trial (mmol/mol)	64 (53–74)	62 (57–73)
(%)	8.0 (7.0-8.9)	7.8 (7.4-8.8)
SD of glucose values (variability) at start of trial	1.8 (1.0 -2.7)	1.7 (1.2 – 2.6)

All values are in median (range) or percentages.
Any micro- or macrovascular complication was defined as the percentage of patients who had one or more complications as judged clinically by the investigator at the moment of inclusion.
BMI = body mass index, DM = diabetes mellitus, SU = sulfonylurea, TZD = thiazolidinediones, BP = blood pressure, HbA1c = glycated haemoglobin, SD = standard deviation

Table 2 Results

	Placebo	Vildagliptin
Primary endpoint		
Units insulin	47 (16 – 62)	34 (12 – 62)
Secondary endpoints		
Glycemic variability end of study (SD)	2.1 (1.1 – 2.8)	1.5 (1.0 – 3.6)
Change in weight (kg)	3 (–2.5 – 5.5)	0.5 (–2.6 – 4)
Hypoglycemia during the study (nr per pat)	1.5 (0 – 5)	1.0 (0 – 8)
Change in blood-pressure (mmHg)		
Systolic	–3 (–12 – 6)	2.5 (–14 – 10)
Diastolic	–1.5 (–9 – 3)	–0.5 (–6 – 11)
Change in LDL (mmol/L)	0 (–1 – 0.6)	–0.3 (–1.4 – 0)
Change in SAF (skin AGEs) (AU)	0.1 (–0.4 – 0.8)	0.15 (–0.4 – 0.6)
Safety		
Patients with one or more hypoglycemia (%)	67%	78%
Patients with severe hypoglycemia (%)	0%	0%
Patients with one or more AE (%)	100%	44%
Patients with one or more SAE (%)	0%	0%
Other		
Delta HbA1c (mmol/mol)	–6.5 (–18 - 7)	–6 (–25 - 4)
(%)	–0.6 (–1.6 – 0.6)	–0.5 (–2.3 - 0.3)
Compliance (% of tablets taken)	96%	98%

All values are in median (range) or percentages. Compliance = % of tablets taken during the whole study period, Delta HbA1c = HbA1c end-begin, –6 means a decrease of 6 points in HbA1c during the study, a number without – means an increase. Because of the small sample size no p-values are shown. LDL = low-density lipoprotein, SAF = skin autofluorescence, AGEs = advanced glycation end products, AU = arbitrary units, AE = adverse event, SAE = serious adverse event, HbA1c = glycated hemoglobin.

Comparable levels of glucose, insulin, C-peptide and glucagon were observed in both groups after mixed meal tests (*Figure 2A-D*). Median values of area under curve (AUC) of glucose and glucagon were 1704 (1439–2279) and 5100 (3030–7620) in the vildagliptin group and 1773 (1439–1931) and 5310 (3525–10785) in the placebo group. Median levels of AUC of insulin and C-peptide were 5295 (1695–24705) and 343050 (106395–963000) in the vildagliptin group and 3795 (1710–7095) and 199703 (134550–281250) in the placebo group. Adverse events were reported more often in the placebo group. These consisted of flu like symptoms (3 patients, all in the placebo group), tiredness (1 vildagliptin, 1 placebo), toothache/ parodontitis (1 vildagliptin, 1 placebo), a cold (1 vildagliptin), headache and diarrhea (1 vildagliptin), reversible increase in gGT (1 placebo, max 165 U/l (normal value: 0–40 U/L)), sensation of tingling in the left arm, which was reversible during the study (1 placebo), mild orthostatic symptoms (1 vildagliptin). None of these events were considered to be severe or related to the study medication.

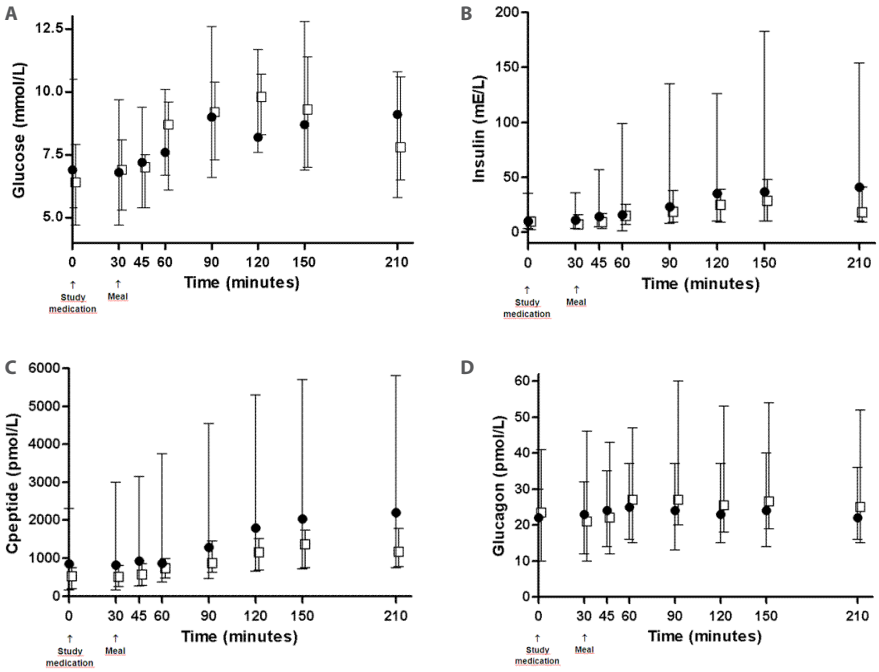

Figure 2 Glucose, insulin, C-peptide and glucagon levels after mixed meal test
☐ = Placebo; ● = Vildagliptin

Discussion

This is the first study investigating the addition of a DPP4-inhibitor to the *start* of once-daily long-acting insulin in insulin-naïve patients. Although this study was set up as a double-blind parallel-arm placebo-controlled trial, the sample size is limited and therefore we cannot make conclusions about the effect of addition of vildagliptin on units of insulin at the end of the study, variability, weight, hypoglycemia or response of insulin, C-peptide or glucagon after a mixed meal test.

This important limitation is due to difficulties in patient recruitment. In the Netherlands, most patients starting with once-daily insulin are treated by a general practitioner and not in an (academic) hospital, where this study was performed. Despite extensive collaboration with many general practitioners and hospitals in the region, patients were still difficult to recruit. The small sample size is therefore too small to have statistical power to confirm or reject the null hypothesis.

However, given the working mechanism of DPP4-inihibitors and our small sample size, we think it would be very interesting to investigate our hypothesis in a larger study. Our small study could potentially serve as a guidance for such larger studies. From our data it can be derived that a repeat study would need approximately 46 patients per group. This is based on a median units of insulin of 40 (SD 17) and 10 units of insulin decrease, which would be clinically significant (and 25% is comparable to the difference in median units of insulin in our study of 28%) . This sample size calculation must be considered as a rough estimation, given the small sample size and not-normal distribution of our main end-point, but at this moment the best estimation available.

Besides the smaller sample size, our study differs in two major design aspects from previous randomized-controlled trials. First, previous trials investigated the effect of add-on DPP4-inhibition therapy to *existing* insulin regimen[4-14], whereas we investigated the addition of vildagliptin to the start of insulin therapy in our study. We hypothesized that adding a DPP4-inhibitor to start of insulin treatment could lead to less exogenous insulin requirements. This could lead to a reduction of hyperinsulinemia, which is thought to have atherogenic and mitogenic effects[21]. Moreover, lower insulin use could reduce side effects of insulin treatment such as hypoglycemia and weight gain[1]. The addition of a DPP4-inhibitor to an existing insulin treatment without the intention to change the insulin regimen as previous trials did does not reflect clinical practice in which physicians will choose to alter insulin schedules. Only in one randomized trial the insulin dose was changed as a goal in one arm (insulin-increasing arm) and compared to the addition of sitagliptin to existing insulin regimen. In that trial, a difference of 25% in insulin dose was described between the insulin-increasing and the insulin-sitagliptin group[9] together with a more pronounced HbA1c decrease in the sitagliptin group. The 25% decrease in units of insulin found in that study is comparable to the magnitude (28%) we found.

Second, we used a different end-point compared to previous trials. In randomized-controlled trials about the effect of the addition of DPP4-inhibitors to insulin thus far, HbA1c or glycemic control were used as primary endpoint[4-14]. We chose required units of insulin since, in our study, we added vildagliptin to the start of insulin and we aimed at the best glycemic control with insulin glargine in all patients.

Because a lower insulin dose may lead to less side effects, we also investigated these as secondary endpoints. The first was hypoglycemia. With improvement of glycemic control, the incidence of hypoglycemic episodes was expected to increase. This was indeed observed in a study using sitagliptin[14]. In contrast, a study by Fonseca et al. showed less hypoglycemia together with a decrease in HbA1c when vildagliptin was added to an insulin regimen[4], although this could not be confirmed in a study by Kothny *et al*., using the same DPP4-inhibitor[10]. Less hypoglycemia, as shown in the study by Fonseca, could be a

result of an improved alpha cell function, which has been described not only postprandial (reduction in glucagon) but also during the reaction after induced hypoglycemia when a slightly increased increment of glucagon was seen[3]. In our study occurrence of hypoglycemia was low in both groups.
The second secondary endpoint studied was weight. In contrast to GLP1-analoga, DPP4-inhibitors do not show a large effect on weight[22]. Differences in body weight were not shown in previous randomized-controlled trials with DPP4 inhibitors in combination with insulin[4-14], except for one study which compared insulin-increasing therapy with addition of sitagliptin to existing insulin regimen[9]. The design of that study resembled ours, and it also found a difference in required units of insulin between the groups. Furthermore, that study showed less weight increase in the DPP4 addition group (between-group difference 1.7 kg in favour of the DPP4-group, which is in concordance in magnitude with our findings (vildagliptin group (+0.5 kg), placebo group (+3 kg)).
We also studied additional endpoints, including glucose variability. We hypothesize that since variability is determined by hyperglycemic and hypoglycemic excursions, effects on alpha cell function could result in less glucose variability. A decrease in postprandial glucagon could reduce hyperglycemic excursions, while an increase in glucagon response following a hypoglycemia could reduce a hypoglycemic excursion. A decrease in variability has been reported before from single-arm trials, investigating the effect of addition of a DPP4-inhibitor to insulin[23, 24] . In our study, median glycemic variability (SD) at the end of the study was 2.1 in the placebo group and 1.5 in the vildagliptin group.
We evaluated responses of insulin, C-peptide and glucagon during a mixed meal test, which were comparable in our study. It is difficult to assess insulin and glucagon dynamics in patients taking insulin[14]. All patients were on stable insulin dose, titrated at the fasting glucose levels and all patients were fasting. However, since exogenous insulin was present at time of testing because all patients took their glargine at bedtime the day before the testing, we cannot exclude that measurements are confounded by exogenous insulin. Since GLP1 could also have an effect on cardiovascular parameters, these were also included as an endpoint[25]. Levels for blood pressure, LDL and skin autofluorescence were comparable.

Conclusions

This small randomized study did not have sufficient power to detect effects of the addition of vildagliptin to the beginning of once daily long-acting insulin. However, in our opinion, adding a DPP4-inhibitor, especially in this group

remains a very interesting approach. This study could be used as a guidance for larger studies to investigate the effects of this intervention on insulin requirements, glycemic variability, hypoglycemia and weight gain.

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Chapter 6

Advanced Glycation End Products, Measured as Skin Autofluorescence and Diabetes Complications, a Systematic Review

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Diabetes Technol Ther. 2011 Jul;13(7):773-9.

Abstract

Background

Advanced glycation endproducts (AGEs) are long-lived tissue proteins that accumulate in diabetes. Skin AGEs measured in biopsy specimens strongly correlated with complications of diabetes. AGEs can also be measured non-invasively by the AGE Reader. The aim of this review was to systematically review all articles on the association between skin autofluorescence (SAF), measured by the AGE-reader and complications of diabetes.

Methods

We screened PubMed for studies on SAF and complications in type 1 and type 2 diabetes. Seven articles met the inclusion criteria.

Results

All studies showed positive associations of SAF with one or more complications (all-cause mortality, cardiovascular mortality, micro- and macrovascular complications, neuropathy and nephropathy), except retinopathy. Only three studies were of prospective design, with a follow-up of 3-5 years; the other four studies were cross-sectional. Studies were of large clinical heterogeneity.

Conclusions

This systematic review of literature showed an association of SAF with end-organ complications in diabetes, except retinopathy, in all seven studies. However, studies were of large clinical heterogeneity, only three studies had a prospective design, and five studies were from the same research group. More prospective studies, with a longer period of follow-up, larger group size, and strict definitions of complications and endpoints, are needed to demonstrate the potential role and benefit in clinical management before the widespread use of the AGE Reader can be recommended.

Introduction

The incidence and prevalence of diabetes are increasing[1-3]. Patients with diabetes can develop microvascular complications, such as retinopathy, nephropathy and neuropathy, and macrovascular complications, including coronary heart disease and cerebrovascular accidents. Many risk factors are known, but all factors together cannot fully explain the risk of diabetes complications. This suggests that other pathophysiological mechanisms are operative. Increased tissue advanced glycation end products (AGEs) may well be such an alternative mechanism.

AGEs are permanently deposited glyco-oxidation products that are formed by different pathways. One is the Maillard reaction, in which glycated proteins are formed by a series of sequential reactions between glucose and proteins[4]; the other is formation by reactive carbonyl compounds, which may form rapidly under oxidative stress[5]. AGE-formation and accumulation can cause damage by two pathways. First, AGEs can form cross-links with proteins that affect the three dimensional structure and thereby the functions of these proteins[6]. Second, AGEs can cause harmful effects by the activation of receptors for AGEs. For instance, stimulation of the receptor for AGEs can lead to activation of second messengers and transcription factors that up-regulate harmful cytokines[7].

AGEs accumulate in the body during aging[8]. This process of accumulation is accelerated in several conditions with glycemic and oxidative stress, resulting in higher AGE levels in, for instance patients with diabetes mellitus, renal failure, patients admitted to the intensive care unit, and patients who smoke[8-11]. AGEs are cleared by the kidney; renal failure results in decreased clearance and thereby AGE-accumulation[12].

AGEs, measured in skin biopsy specimens, are positively associated with the presence and severity of microvascular disease in patients with diabetes[13-15]. In prospective studies in diabetes patients, the level of skin AGEs, measured in biopsy specimens, predicts the progression of microvascular complications (retinopathy and nephropathy), and the level of serum AGEs predicts mortality rate (all-cause and cardiovascular)[16, 17]. However, all these studies use invasive methods to measure AGEs, limiting clinical implementation of assessing AGE-accumulation.

The level of AGEs in the skin can also be measured non-invasively by the AGE Reader™ (DiagnOptics B.V., Gronigen, The Netherlands), formerly known as the AFR (Autofluorescence reader)[18]. This noninvasive method uses skin autofluorescence (SAF) and is based on the specific fluorescence characteristics of some AGEs. SAF is validated against AGE levels in skin biopsy specimens (both fluorescent and nonfluorescent AGEs) in healthy controls, patients with

diabetes, and patients on hemodialysis[19, 20]. Because the AGE Reader allows noninvasive estimations of skin AGEs, this method could be of clinical potential to predict diabetes complications and indeed can be purchased for this purpose. However, a systematic review on the current literature investigating the association of SAF, measured by the AGE Reader with diabetes complications is lacking. Therefore we performed this systematic review to critically assess the current evidence for the potential of the AGE Reader to be used as a tool in daily clinical practice.

Subjects and Methods

Literature search

A literature search was carried out in PubMed to identify all relevant studies up to June 2010 regarding the association of skin AGEs, measured noninvasively by the AGE Reader or AFR, and complications in patients with diabetes mellitus. We used the search terms as mentioned in *Table 1*; search terms within the columns were connected with "or", and finally the columns were connected with "and" to yield our final search. Only original articles in English were included. Studies on the same topic in the reference lists of the reviewed articles were also retrieved.

Inclusion criteria

Any study that met the following criteria was included in this review:
- patients with type 1 or type 2 diabetes
- SAF levels measured by the AGE Reader or AFR
- information concerning complications of diabetes

Outcomes of interest

The following subjects were classified as outcomes of interest:
- all-cause mortality
- cardiovascular mortality;
- macrovascular complications;
- microvascular complications;
- separate microvascular complications
    - neuropathy
    - nephropathy
    - retinopathy

Table 1 Search strategy

Subjects	and	Method of measurement	and	Outcome
Diabetes		skin advanced glycation endproducts		prediction
Mellitus				
Diabetes		skin AGEs		predictive value complications
DM		AGE-reader		cardiovascular complications
Diabetic patients		AGE reader		
Diabetic subjects		AFR		mortality
		autofluorescence reader		macrovascular complications
		skin autofluorescence		macro-vascular complications
		autofluorescence		microvascular complications
		SAF		micro-vascular complications
				retinopathy
				nephropathy
				neuropathy

Search terms within the columns were connected with "or," and the searches resulting from the columns were connected with "and" to yield our final search.

Statistics

Studies included a multitude of different patient groups and investigated different outcomes, as described in *Table 2*; therefore a meta-analysis could not be performed due to clinical heterogeneity.

All results and statistics in different studies are described in *Table 3*. The studies included used different statistics to describe outcome. Most cross-sectional studies described differences in SAF between patients with and without diabetes complications[21, 22]. One study used a β-coefficient[23]. Two studies described a hazard ratio for developing complications[24, 25] and two used odds ratios (ORs)[26, 27].

Different studies adjusted effects of SAF for different factors (Suppl 1). Chabroux *et al*.[23] adjusted for age, diabetes' duration, glycated haemoglobin (HbA1c), smoking, retinopathy, nephropathy and neuropathy. Lutgers *et al*.[21] adjusted only for age. Meerwaldt *et al*.[22] corrected for HbA1c, age, diabetes' duration, serum creatinine and microalbuminuria in their analysis of the association of SAF with nerve conduction velocity. However, adjustments of differences in SAF between patients with and without neuropathy were not explicitly described. Monami *et al.*[24] adjusted for age and HbA1c. ORs mentioned in the

Table 2 Studies and characteristics of participants

Study, country (year)	Design	Outcome	Number (n)	Age (years)
Chabroux et al.[23] France (2010)	Cross-sectional	Neuropathy, nephropathy, retinopathy	T1: 133	T1: 30 (23)
Lutgers et al.[21], The Netherlands (2006)	Cross-sectional	Macrovascular, microvascular	T2: 973 C: 231	T2: 66 ± 11 C: 52 ± 17
Meerwaldt et al.[22] The Netherlands (2005)	Cross-sectional	Neuropathic foot ulceration	T1 and T2 NP+ subjects: 24	57 ± 12
			T1 and T2 NP– subjects: 23	53 ± 13
			C: 21	58 ± 10
Monami et al., Italy (2008)[24]	Cross-sectional	Macrovascular (arteriopathy of the lower limbs, ischemic heart disease, stroke/TIA), neuropathy, foot ulceration, nephropathy, retinopathy	T2: 92	T2: 69.1 ± 12.4
Gerrits et al.[26] The Netherlands (2008)	Prospective, follow-up 3.1 years	Microvascular (neuropathy, nephropathy, retinopathy)	T2: 881	T2: 66 ± 11
Lutgers et al.[25] The Netherlands (2009)	Prospective, follow-up 3.1 years	All-cause mortality, cardiovascular events	T2: 967	T2: 66 ± 11
Meerwaldt et al.[27] The Netherlands (2007)	Prospective, follow-up 5 years	Cardiovascular mortality (prospective), coronary heart disease (cross-sectional)	T1: 48 T2: 69 C: 43	T1: 45 ± 15 T2: 61 ± 13 C: 53 ± 16

Data are expressed as mean ± SD or median (interquartile range [IQR]).
C = control subjects without diabetes; DM = diabetes mellitus; HbA1c = glycated hemoglobin; NP+ = diabetes patients with neuropathic foot ulceration, NP– = diabetes patients without clinical neuropathy;
T1 = type 1 diabetes; T2 = type 2 diabetes; TIA = transient ischemic attack.

follow-up study of Gerrits *et al.*[26] are adjusted for sex, body mass index, HbA1c, diabetes' duration, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, smoking and hypertension but not age. Results described by Meerwaldt *et al.*[27] are adjusted for age, body mass index, HbA1c, diabetes' duration, triglycerides, low-density lipoprotein cholesterol, smoking, hypertension, plasma creatinine, hemodialysis treatment and coronary heart disease at baseline. Hazard ratios mentioned in the follow-up study of Lutgers *et al.*[25] are adjusted for age, diabetes' duration, sex, smoking, HbA1c, systolic blood pressure, total cholesterol and high-density lipoprotein cholesterol.

DM duration (years)	HbA1c (%)	Therapy	History of complications (% of diabetes patients)
T1: 17 (15)	T1: 8.0 (1.5)	100% insulin	41% microvascular disease: 9% neuropathy, 18% nephropathy, 40% retinopathy
T2: 4.2 (1.6–8.3)	T2: 7.0 ± 1.3	16% insulin, 84% non-insulin treatment	39% macrovascular disease 49% microvascular disease: 28% neuropathy, 21% nephropathy, 20% retinopathy
17 ± 12	8.2 ± 1.09	75% insulin, 25% non-insulin treatment	17% coronary heart disease: 100% neuropathy, 88% retinopathy
13 ± 10	7.6 ± 0.85	83% insulin, 17% non-insulin treatment	
	5.9 ± 0.72		
T2: 12.3 ± 10.7	T2: 7.6 ± 1.4	36% on insulin, 64% non-insulin treatment	Macrovascular disease: 28.3% ischemic heart disease, 14.1% stroke/TIA Microvascular disease: 30.4% neuropathy, 23.0% foot ulcers, 19.6% microalbuminuria, 8.7% retinopathy
T2: 4.0 (1.5–8.1)	T2: 6.6 (6.0–7.6)	15% insulin, 85% non-insulin treatment	37% macrovascular disease, 50% microvascular disease (24% neuropathy, 24% nephropathy, 19% retinopathy)
T2: 4.2 (1.6–8.3)	T2: 7.0 ± 1.3	16% insulin, 84% non-insulin treatment	39% macrovascular disease, 53% microvascular disease (29% neuropathy, 25% nephropathy, 20% retinopathy)
T1: 20 ± 11 T2: 10 (5–20)	T1: 7.9 ± 1.0 T2: 8.2 ± 0.9 C: 5.5 ± 0.05	No information about insulin or oral therapy is given	T1: 27% coronary heart disease, 21% end-stage renal failure (on hemodialysis) T2: 45% coronary heart disease, 20% end-stage renal failure (on hemodialysis)

Results

Characteristics of studies

In total, 141 papers were found. After title and abstract were screened, 134 papers were about different topics and were excluded. Seven papers were included: four[21-24] were cross-sectional studies, and three[25-27] were prospective studies. Five of the seven studies were from one research group from Groningen, The Netherlands. Two of the investigators from this group were founders of the company providing the AGE Reader. Characteristics of studies and study

participants are described in *Table 2*. It must be noted that studied populations were very heterogeneous. Studies included subjects with different types of diabetes, treatment, diabetes' duration, and the participants included were of different ages, with a different history of complications. Studies investigated different outcome (different diabetes complications). The follow-up study of Lutgers *et al.*[25] used the same cohort as the cross-sectional study of Lutgers *et al.*[21], except for six patients who were lost to follow-up. Gerrits *et al.*[26] also used data from this cohort of patients (a study in a large primary care population of patients with type 2 diabetes). Five studies used the AFR to measure SAF (prototype of the current AGE Reader)[21, 22, 25-27] and two studies used the AGE Reader.[23, 24] Different studies adjusted results for different factors (*Suppl 1*); it is notable that Gerrits *et al.*[26] did not adjust for age on multivariate analysis. Three of the seven studies compared SAF in diabetes patients with SAF in control subjects without diabetes[21, 22, 27]. In these three studies SAF was higher in the diabetes group.

Outcome

A summary of outcomes of all studies is mentioned in *Table 3*.

All-cause mortality

The only study investigating this end-point[25] showed a positive association of SAF with all-cause mortality in type 2 diabetes patients on multivariate analysis (HR of 2.05 [95% confidence interval (CI) 1.22-3.45] with a median follow-up of 3.1 years).

Cardiovascular mortality

The only study investigating this end-point[27] found a positive association of SAF with cardiac mortality on multivariate analysis with 5 years of follow-up (OR of 2.00 [95% CI 1.3-2.7] in type 1 diabetes, and 2.9 [95% CI 1.3-4.4] in type 2 diabetes). The area under a receiver operating characteristic curve using SAF to detect mortality was significantly higher than similar curves using HbA1c (SAF 0.92 vs. HbA1c 0.82 [type 1 diabetes] and 0.61 [type 2 diabetes]).

Macrovascular complications

Four studies investigated this endpoint. Three of these studies found some association of SAF with macrovascular complications. One study[21] showed a significantly higher SAF in diabetes patients with macrovascular complications compared with diabetes patients without complications. They also found a significantly higher SAF in diabetes patients with concomitant micro- and

macrovascular complications compared to diabetes patients with only microvascular complications and patients without complications. Meerwaldt *et al*.[27] showed a positive association of coronary heart disease with SAF at their cross-sectional part of the study as well (OR of 7.8 in type 1 diabetes and 7.9 in type 2 diabetes [no CIs were given]). Monami *et al*.[24] demonstrated a positive association of SAF with arteriopathy of the limbs, whereas SAF was not associated with ischemic heart disease and transient ischemic attack/ stroke. The only prospective study investigating this endpoint[25] did not find a significant association of SAF with cardiovascular events (fatal and non-fatal).

Microvascular complications

The two studies investigating this endpoint were of different design (cross-sectional/ prospective) and showed different results. Lutgers *et al.*[21] found no significant difference in SAF between patients with microvascular complications and patients without complications. In the prospective study of Gerrits *et al*.[26], however, SAF was a strong predictor of the development of microvascular complications on multivariate analysis (OR of 2.02 [95% CI 1.51-2.80]). Of note is that both studies used data from the same cohort of patients; one described association of SAF and complications cross-sectionally, and the other investigated partly the same patients after a follow-up of 3.1 years.

Different microvascular complications:

Neuropathy. The four studies investigating this endpoint all show a positive association of SAF with neuropathy. One study had a prospective design[26] (OR of 1.50 [95% CI 1.05-2.14]) and three studies had a cross-sectional design[22-24]. Meerwaldt *et al.* demonstrated a significantly higher SAF in diabetes patients with a history of neuropathic foot ulceration compared with diabetes patients without clinical neuropathy. In this study SAF also correlated negatively with both sensory and motor nerve conduction velocities and amplitude. Besides the association with neuropathy, Monami *et al.*[24] found a positive association of SAF with current foot ulceration as well.

Nephropathy. Three studies investigated this endpoint, and all showed some positive result. One[26] showed an OR of 1.88 (95% CI 1.36-2.61) for developing nephropathy with each increment of SAF. Chabroux et al.[23] also showed an association between SAF and incipient nephropathy (defined as micro-albuminuria) as well as overt nephropathy (macro-albuminuria). The third study[24] did not show a significant association for microalbuminuria, but it did for chronic renal failure.

Retinopathy. Neither the cross-sectional[23,24] nor the prospective study[26] could demonstrate a significant association of retinopathy with SAF.

Table 3 Results and Statistics

Study, type (device)	Number	Statistical analysis, expressed as	Reference group	Mortality
Chabroux et al.,[23] cross-sectional (AGE Reader)	T1: 133	β-coefficient (95% CI)	T1 subjects without neuropathy T1 subjects without nephropathy T1 subjects without retinopathy	
Lutgers et al.,[21] cross-sectional (AFR)	T2: 973 C: 231	Mean ± SD SAF or mean SAF (95% CI)	T2 subjects without complications: 2.57 (2.50–2.65) Control: 2.14 ± 0.6	
Meerwaldt et al.,[22] cross-sectional (AFR)	T1 and T2 NP+ subjects: 24 T1 and T2 NP− subjects: 23 C: 21	Mean ± SD SAF	Control: 0.011 ± 0.001	
Monami et al.,[24] cross-sectional (AGE Reader)	T2: 92	HR (95% CI)	Each unit of increment of SAF	
Gerrits et al.,[26] prospective (AFR)	T2: 881	OR (95% CI)	Each unit of increment of SAF	
Lutgers et al.,[25] prospective (AFR)	T2: 967	HR (95% CI)	Below or above median SAF	**All-cause mortality HR 2.05 (1.22–3.45)**
Meerwaldt et al.,[27] prospective (AFR)	T1: 48 T2: 69 C: 43	OR (95% CI)	Each unit of increment of SAF	**Prospective: cardiovascular mortality: T1 OR 2.00 (1.3–2.7) T2 OR: 2.9 (1.3–4.4)**

All significant results (significance level: $P < 0.05$) are given in **bold** type; all results that are not significant are in *italic*.

Statistics: compared with diabetes patients [*]without complications and [a]without neuropathy, [b]without nephropathy, or [c]without retinopathy; [†]control subjects without diabetes; or [‡]diabetes patients with microvascular complications. All values mentioned are adjusted for confounding factors.

AFR = autofluorescence reader; C = control subjects without diabetes; CI = confidence interval; HR = hazard ratio; NP+ = diabetes patients with neuropathic foot ulceration, NP− = diabetes patients without clinical neuropathy; OR = odds ratios; T1 = Type 1 diabetes; T2 = type 2 diabetes; TIA = transient ischemic attack.

Macrovascular complications	Microvascular complications	Neuropathy	Nephropathy	Retinopathy
		Neuropathy: 0.46 (0.02–0.91)[a]	**Incipient nephropathy: 0.37 (0.03–0.70)[b]** **Overt or renal failure: 0.58 (0.18–0.99)[b]**	*Moderate retinopathy: 0.08 (−0.14 to 0.30)[c]* *Severe retinopathy: 0.12 (−0.17 to 0.42)[c]*
T2 macrovascular complications: 2.91 (2.78–3.03)[*,†] **T2 micro- and macrovascular complications: 3.12 (3.01–3.23)[*,‡]**	T2 microvascular complications: 2.71 (2.62–2.80)[*,‡]			
		NP+: 0.025 ± 0.007[a,†]		—
		NP−: 0.017 ± 0.004[†]		
Arteriopathy of the limbs HR 1.9 (1.1–3.6)		**Neuropathy HR 2.1 (1.1–3.8)**	*Microalbuminuria HR 1.4 (0.8–2.5)*	*Retinopathy HR 2.1 (0.9–4.6)*
Ischemic heart disease HR 1.6 (0.9–2.7) *Stroke/TIA HR 1.4 (0.7–2.7)*		**Current foot ulcers HR 3.4 (1.6–7.3)**	**Chronic renal insufficiency HR 2.4 (1.1–5.4)**	
	OR 2.02 (1.45–2.81)	**OR 1.50 (1.05–2.14)**	**OR 1.88 (1.36–2.61)**	*T2 OR 1.21 (0.83–1.74)*
Cardiovascular events HR 1.46 (0.97–2.20)				
Cross-sectional: T1 OR 7.8 T2 OR 7.9 (no CIs were given)				

Discussion

In this first systematic review on SAF as a non-invasive measure of accumulation of AGEs and diabetes complications we found that all eligible studies showed a positive association between SAF and one or more diabetes complications (all-cause mortality, cardiovascular mortality, micro- and macrovascular complications, neuropathy and nephropathy), with the exception of retinopathy. However, these results should be interpreted with caution, because the number of studies is limited and there is large heterogeneity between the studies.

The risk of developing micro- and macrovascular complications is not fully explained by the currently established risk factors. Apparently, unknown factors play a role in the pathogenesis of complications; AGEs may be one of them[28]. The three prospective studies included in this review showed a significant association of the level of SAF at baseline and development of microvascular complications and the all-cause and cardiovascular mortality rate, even after adjusting for possible confounding factors. Moreover; using a different approach, one of these studies[25] also showed that SAF provided additional information to the United Kingdom Prospective Diabetes Study risk score for the estimation of cardiovascular prognosis in type 2 diabetes. Therefore, SAF might be a promising tool to improve the prediction of complications in the long term.

The association of SAF with diabetes complications does not necessarily mean that this is a causal relationship or that interventions aiming at decreasing SAF will lead to a reduction in chronic organ complications. AGEs have several deleterious effects, such as affecting proteins by changing their three-dimensional structure and activating second messengers and transcription factors by stimulating the receptor for AGEs[6, 7, 28]. Therefore, it is possible that AGEs not only predict complications but also are a cause of complications. To investigate this, intervention trials with agents that decrease AGE accumulation are required. Multiple studies in animal models have already shown that pharmacological intervention in AGE accumulation has the potential to alleviate end-organ damage[29]. However, evidence from clinical studies (in humans), supporting this phenomenon is lacking[29].

None of the studies showed any association between SAF and retinopathy. In contrast, the Diabetes Control and Complications Trial-Epidemiology of Diabetes Interventions and Complications (DCCT-EDIC) study[16] did demonstrate that AGEs, measured in skin biopsies specimens, can predict the progression of diabetic retinopathy in type 1 diabetes patients. The discrepancy between these results could be explained by the differences in participants (type of diabetes), differences in duration of the studies (3.1 years in Gerrits *et al*.[26] versus a follow-

up of 10 years in the DCCT-EDIC study[16]), the difference in the percentage of patients developing (progression of) retinopathy (7% vs. >30%), or differences in defining the outcome (Gerrits *et al.*[26] investigated the development of retinopathy [defined by the presence of at least background retinopathy], whereas the DCCT-EDIC study[16] investigated progression of retinopathy [defined by a worsening of three or more steps on a diabetic retinopathy scale]). Finally possible differences in pathogenesis between retinopathy and other complications could play a role.

Several study limitations of this review must be noted. First, only seven studies have been published on SAF and complications, and five of these studies are from the same research group (Groningen, the Netherlands). Also, most studies included only small numbers of subjects. Second, only three studies had a prospective design, assessing the predictive value of SAF, instead of a cross-sectional design, in which only an association can be investigated. Third, studies investigated different and mostly multiple outcomes. Most single outcomes were only investigated in one to four separate studies. Also differences in SAF found in the seven studies were not adjusted for the same factors. In fact, one study even did not adjust for age, a factor, known to be strongly associated with SAF. The definitions of the outcomes were different, using a variety of diagnostic tools. For example, Meerwaldt *et al*[22] defined the presence or absence of neuropathy by the Dutch Diabetic Neuropathy Symptoms scale and the Dutch Diabetic Neuropathy Examination scale, whereas Chabroux *et al*[23] defined neuropathy by only using a monofilament and a graduated tuning fork.

Conclusions

This systematic review of literature showed an association of SAF with end-organ complications in diabetes, except retinopathy, in all seven studies. However, studies were of large clinical heterogeneity, only three studies had a prospective design and five studies were from the same research group. More prospective studies, with longer period of follow-up, larger group size, and strict definitions of complications and endpoints, are needed to demonstrate the potential role and benefit in clinical management before the widespread use of the AGE-reader can be recommended.

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Supplement 1 Different Studies Adjusted Effects of Skin Autofluorescence for Different Factors

	Adjusted effects in the given study						
	Chabroux et al.[23]	Lutgers et al.[21]	Meerwaldt et al.[22]	Monami et al.[24]	Gerrits et al.[26]	Lutgers et al.[25]	Meerwaldt et al.[27]
Age	x	x	x[a]	x		x	x
Sex					x	x	
BMI					x		x
Diabetes' duration	x		x[a]		x	x	x
HbA1c	x		x[a]	x	x	x	x
Smoking	x				x	x	x
Plasma creatinine			x[a]				x
Hemodialysis							x
Coronary heart disease at baseline							x
Blood pressure					x	x	x
HDL-cholesterol					x	x	
LDL-cholesterol					x		x
Total cholesterol						x	
Triglycerides					x		x
Neuropathy	x						
Nephropathy	x						
Retinopathy	x						
Microalbuminuria			x[a]				

[a] Results for the analysis of the association of skin autofluorescence with nerve conduction velocity were corrected for glycated hemoglobin (HbA1c), age, diabetes' duration, serum creatinine, and microalbuminuria. Corrections for the same factors for differences in skin autofluorescence between patients with and without neuropathy were not clearly described.
BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Chapter 7

Accumulation of Advanced Glycation End Products, Measured by Skin Auto-fluorescence is Associated with Presence and Number of Diabetes Complications; a Cross-sectional Study

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Submitted

Abstract

Background

Experimental studies suggest a pathophysiological role for accumulation of advanced glycation end products (AGEs) in diabetes complications. Studies in humans show association of AGE accumulation, measured as skin auto-fluorescence (SAF), with different complications, but results are contradictory, especially for retinopathy. Furthermore, most studies did not investigate all different complications in one study, while in this study we did.

Methods

We performed a cross-sectional study in our university medical centre. SAF was measured in patients with type 2 diabetes by the AGE-reader. We defined neuropathy as detection of vibration ≥ 25 Volts, retinopathy as any degree of diabetes retinopathy by fundus photography, nephropathy as MDRD < 60 ml/min/1.73m^2, peripheral artery disease (PAD) as ankle brachial index (ABI) < 0.8 and macrovascular complications as myocardial infarction, cerebrovascular accident, vascular surgery or angioplasty.

Results

Elevated skin auto-fluorescence was associated with increasing number of microvascular complications (ß 0.16, p=0.019), neuropathy (OR 1.6 (1.1–2.4)), nephropathy (OR 1.9 (1.2-2.9)), PAD (2.0 (1.1-3.6) worse values at neurothesiometer (p=0.001), MDRD (p=0.002) and ABI (p=0.047-0.111), all after correction for confounders. SAF was associated with proliferative retinopathy (OR 3.3 (1.4-7.7)) but not with non-proliferative retinopathy. SAF was not significantly associated with macrovascular complications, after adjustment for confounders.

Conclusions

Accumulation of AGEs, measured by skin autofluorescence is associated with presence and number of diabetes complications in patients with type 2 diabetes. The clinical implication of these associations awaits intervention studies.

Introduction

Advanced glycation end products (AGEs) accumulate in body tissue during aging. Accelerated accumulation is seen with glycemic or oxidative stress and with decreased renal clearance, resulting in AGE accumulation in for instance patients with diabetes mellitus, patients admitted to the intensive care unit and patients with renal failure[1-3]. In patients with diabetes, AGEs in skin biopsies predict the progression of microvascular complications (retinopathy and nephropathy), and the level of serum AGEs predicts mortality rate (all-cause and cardiovascular)[4, 5]. However these studies use invasive methods to measure AGEs, whereas AGE-accumulation can also be assessed non-invasively by the AGE-reader[6]. This method uses skin auto-fluorescence (SAF) and is validated against AGE levels in skin biopsy specimens (both fluorescent and non-fluorescent AGEs) in healthy controls, patients with diabetes, and patients on hemodialysis[7, 8].

In a previous review about the AGE-reader we described that all seven eligible studies showed a positive association between SAF and one or more diabetes complications with the exception of retinopathy[9]. Data on retinopathy are contradictory with more recent studies showing a positive association of SAF with retinopathy[10-12]. Studies investigating the association between SAF and diabetes complications so far used different definitions and different (number of) complications as endpoints, whereas most studies did not investigate all different micro- and macrovascular complications in one study.

In this study we aimed to comprehensively investigate the association of SAF with both micro- and macrovascular complications, including retinopathy in a group of patients with type 2 diabetes in our university medical centre. All complications were measured in a standardized way at the time SAF was measured and defined as dichotomous as well as continuously described endpoints.

Materials and Methods

Study design and patients

This observational prospective study was conducted at the University Medical Centre Utrecht (UMCU), the Netherlands. All patients in this study participated in a nationwide long-term biobank initiative in patients with type 2 diabetes[13]. The current study was a local add-on to this national database study. The study was conducted according to Good Clinical Practice. The protocol was approved by the ethics committee of the University Medical Centre Utrecht. Written

informed consent was obtained from all patients prior to measurements. Patients with type 2 diabetes attending the (outpatient) clinic of the UMCU were approached to participate in this study and included in this study from February 2010 till July 2013. All patients with a diagnosis of type 2 diabetes were eligible for the study.

Skin autofluorescence (AGE-reader)

The level of AGEs in the skin was measured non-invasively by the AGE-reader (DiagnOptics Technologies BV, Groningen, The Netherlands). The AGE-reader is a desktop unit on which the patient positions the volar side of the right lower arm on a light source. The excitation light source is an ultraviolet-A black light tube, with a wavelength between 350 and 420-nm (peak wavelength of 370 nm), which illuminates around 2 cm^2 of the skin. A spectrometer detects the reflected light from the skin in the 420- to 600-nm range. SAF is calculated as the ratio of the total emission intensity and the total excitation intensity and expressed in arbitrary units (AU). The measurements of SAF are validated against AGE-levels in skin biopsies in healthy controls, in patients with diabetes and in patients on haemodialysis[7, 8]. Prior reproducibility studies of repeated measurements in healthy volunteers have shown a mean relative error of 5%[14, 15]. The AGE-reader has been validated in patients with skin reflectance ≥6%. A correction is made to the SAF-value if the reflectance is between 6 and 12%. If the reflectance is below 6%, mostly in patients with a dark brown and black skin, measurement with the AGE-reader is not possible.

Assessment of complications

All patients filled in health questionnaires and visited the outpatient clinic for assessment of complications, SAF and laboratory measurements. Vibration sense was measured using a neurothesiometer. This device was applied to the apex of both big toes three times and a mean of the perceptive voltage was calculated. A level of 25 Volt or more was considered to represent diminished vibration sense, indicating presence of diabetic neuropathy.
Retinopathy was assessed from digital fundus photographs taken following standard procedure. After pupil dilatation 7 photographs were taken covering the macula, optic nerve and peripheral fields and retinopathy was assessed in one overlay photo.
Ankle brachial index (ABI) was calculated from the blood pressure measured at each foot, divided by the highest blood pressure of one of the arms. An ABI of > 1.3 was considered unreliable and an ABI of ≤ 0.8 was considered a strong indication of peripheral artery disease.

Definition of endpoints

Diabetes complications were defined before start of the study. We defined dichotomous endpoints for analysis as follows. Neuropathy was defined as a detection of vibration at 25 Volts or more with neurothesiometer (left or right foot). Retinopathy was defined as any abnormality observed at fundus photographs consistent with diabetes retinopathy. Nephropathy was defined as a GFR (MDRD) of < 60 ml/min/173m^2. Macrovascular complications were defined as a composite endpoint of myocardial infarction (MI), cerebrovascular accident (CVA), vascular surgery or angioplasty (coronary, carotid, femoral, iliacal or aortic). Peripheral artery disease (PAD) was defined as an ABI < 0.8 measured at the right or left lower extremity.
For exploratory analysis we also used crude values at neurothesiometer, MDRD and ABI, as well as degree of retinopathy. Neurothesiometer values range from 0 to 50 (highest possible), if 50 volt was not felt by the patient a value of 51 volt was used. For continuous analysis of MDRD, only patients with MDRD <= 60 ml/min/173m^2 were included (n=80). For analysis of ABI patients with reliable ABI (<1.3) were selected (left n=296, right n=285). For exploratory analysis proliferative retinopathy was defined as abnormalities at fundus photography consistent with proliferative retinopathy or a history of laser coagulation (n=9). All other abnormalities consistent with diabetes retinopathy were scored as non-proliferative retinopathy.

Statistical analysis

Results are presented as mean and SD for normally-distributed variables and median and range for not-normally distributed parameters. Normality was tested by QQ plots. If data were not-normally (and right-skewed) distributed, log-transformation was performed. Base-line characteristics of included patients were compared to non-included patients. A student's t-test was used to compare between two groups with normal distribution and ANOVA with Bonferroni to compare multiple groups. We analysed the association of SAF with pre-defined dichotomous endpoints, but we also used continuous endpoints as an exploratory analysis (crude values on neurothesiometer, MDRD, ABI). For the analysis of increasing number of microvascular complications we used all patients with available information on all microvascular complications (n=309). Linear and logistic regression models (univariate and multivariate) were used to investigate relations between SAF and different diabetes complications. Relation of SAF with endpoints was first analysed univariately. Influence of potential confounders was tested in a multivariate model with SAF and a confounder. Missing values of confounders (age and sex complete, HbA1c 1

missing value, duration of diabetes 20 missing values) were imputed, using the mean value. Age was the most important confounder for neuropathy, nephropathy and macrovascular complications. Therefore multivariate models were described separately adjusted for age. Finally in a multivariate model we adjusted for all predefined confounders (age, sex, duration of diabetes). We also tested influence of HbA1c on OR for different outcomes by including it in the final model. In a sensitivity analysis we adjusted for MAP (for nephropathy and macrovascular disease) and smoking (for macrovascular disease). All these analyses were also adjusted for autoimmune disease and recent infection or hospital admission in a sensitivity analysis, since the literature suggest higher SAF levels in patients with autoimmune disease or with recent infection[15-18]. Level of significance was set at $P< 0.05$. A sample size calculation showed that with an alpha of 0.05 and a power of 80% we would need 242 patients to show a difference of 15% in complications.

Results

We screened 1372 patients and 508 patients were included, 5 withdrew consent, leaving 503 patients for analysis. Since the AGE-reader was at the time used in our centre for multiple studies it was not always available for measurements. SAF measurements in this study were eventually performed in 343 patients.

Baseline characteristics are shown in *Table 1*. Included patients were predominantly of Caucasian origin, their mean age was 61 years, BMI 31 kg/m^2, HbA1c 58 mmol/mol (7.5%). Mean duration of diabetes was approximately 12 years and slightly more than half of the patients (55%) used insulin. Characteristics of patients that could not be included were comparable (*Table 1*), apart from the fact that included patients were slightly older, more of Caucasian ethnicity and had slightly more nephropathy.

Higher SAF levels were associated with neuropathy, nephropathy, PAD and macrovascular complications in crude models (*Table 2*). For neuropathy, nephropathy and PAD these associations remained significant after adjustment of confounders. Adjustment for HbA1c did not alter these associations. For macrovascular complications the association with SAF attenuated to non-significant when adjusted in the multivariate model. SAF was not at all associated with diabetic retinopathy (defined as any degree of diabetic retinopathy).

Elevated SAF was associated with proliferative retinopathy, both compared to patients without retinopathy as well as compared to non-proliferative retinopathy. This association persisted when adjusted for age (*Table 3*).

Elevated SAF levels were also linearly associated with worse values measured with neurothesiometer and MDRD, both in the crude and multivariate analyses (*Table 4*). Adjustment for HbA1c did not alter these associations.

Table 1 Baseline characteristics of the 343 patients with type 2 diabetes included versus patients that could not be included

	Included patients (n=343)	SAF not measured, not included (n=160)
Age (years)	61 (11)	58 (12)*
Sex (% female)	40%	39%
Ethnicity (% Caucasian)	90%	78%*
BMI (kg/m^2)	31 (6)	32 (6)
HbA1c (mmol/mol)	58 (15)	58 (16)
Duration of diabetes (years)	12 (10)	12 (10)
Mean arterial pressure (MAP)	98 (11)	98 (13)
Current smoking (self-reported)	16%	20%
Pack years	14 (0-165)	16 (0-156)
Complications		
microvascular		
neuropathy	29%	29%
retinopathy	22%	27%
nephropathy	23%	13%*
macrovascular	37%	37%
Therapy		
None	5%	4%
Oral agents only/ GLP-1 analoga	40%	38%
Insulin	55%	58%
Long-acting	5%	7%
Premix	7%	8%
Intensive	37%	33%
CSII	6%	9%

Parameters are expressed as mean (SD) or median (range) or %
HbA1c 61 mmol/mol equals 7.7%, HbA1c 58 mmol/mol equals 7.5%

Table 2 Logistic regression models for the relation between SAF and certain diabetes complications

	Neuropathy	Nephropathy	Retinopathy	Macrovascular	PAD
SAF	2.3 (1.6 – 3.3) p<0.001	2.7 (1.8-4.0) p<0.001	1.3 (0.9-2.0) p=0.163	1.9 (1.3-2.6) p<0.001	2.5 (1.4-4.2) p=0.001
SAF age-adjusted	1.7 (1.1 – 2.5) p=0.010	1.9 (1.3-2.9) p=0.002	1.2 (0.8-1.9) p=0.365	1.4 (1.0-2.0) p=0.073	2.1 (1.2-3.7) p=0.012
SAF MV	1.6 (1.1 – 2.4) p=0.021	1.9 (1.2-2.9) p=0.003	1.0 (0.9-1.0) p=0.924	1.3 (0.9-1.9) p=0.130	2.0 (1.1-3.47) p=0.017
SAF MV + HbA1c	1.6 (1.1 – 2.4) p=0.022	1.9 (1.2-2.9) p=0.003	1.0 (0.6-1.6) p=0.857	1.2 (0.9-1.9) p=0.134	2.0 (1.1-3.46) p=0.020

Data shown are odds ratio (OR) and (95% confidence interval (CI)), p-value
SAF = univariate regression with SAF as only variable; SAF age-adjusted = adjusted for age; SAF MV = multivariate regression adjusted for pre-defined confounders (age, sex, duration of diabetes); SAF MV + HbA1c = adjusted also for HbA1c; PAD = peripheral artery disease

Table 3 Association of SAF with proliferative retinopathy

	Proliferative retinopathy	
	No retinopathy	Non-proliferative
SAF	3.47 (1.56-7.76)	3.19 (1.34-7.60)
SAF age-adjusted	3.32 (1.44-7.68)	3.39 (1.35-8.49)
SAF MV	-	-
SAF MV + HbA1c	-	-

Data are presented as OR (95% CI)
SAF = univariate regression with SAF as only variable; SAF age-adjusted = adjusted for age; (-) Analysis not performed for proliferative retinopathy since n was 9

Table 4 Association of SAF with (log)values measured by neurothesiometer, MDRD and ankle-brachial index

	Neurothesiometer		MDRD	Ankle brachial index	
	Left	Right		Left	Right
SAF	0.38 (p<0.001)	0.33 (p<0.001)	-5.9 (p<0.001)	-0.05 (p=0.001)	-0.04 (p=0.007)
SAF age-adjusted	0.24 (p<0.001)	0.19 (p<0.001)	-5.8 (p<0.001)	-0.03 (p=0.029)	-0.03 (p=0.093)
SAF MV	0.21 (p<0.001)	0.18 (p=0.001)	-5.0 (p=0.002)	-0.03 (p=0.041)	-0.03 (p=0.091)
SAF MV + HbA1c	0.21 (p<0.001)	0.17 (p=0.001)	-5.0 (p=0.002)	-0.05 (p=0.047)	-0.03 (p=0.111)

Data are presented as ß (p-value) Data for neurothesiometer and ankle brachial index were log-transformed for analysis
SAF = univariate regression with SAF as only variable; SAF age-adjusted = adjusted for age; SAF MV = multivariate regression adjusted for pre-defined confounders (age, sex, duration of diabetes); SAF MV + HbA1c = adjusted also for HbA1c

Elevated SAF levels were also related to lower ABI levels, although this association partly lost significance when adjusted for confounders.
Mean SAF in this population was 2.8 AU (0.7). This is elevated (p<0.001) when compared to SAF in subjects without diabetes of comparable age (2.34 AU)[19]. Mean SAF in patients with diabetes complications was significantly higher compared to SAF in patients without these complications, except for retinopathy (*Table 5A*). Mean SAF in patients with microvascular complications increased with increasing number of complications in both crude (p< 0.001) and multivariate analysis (age, sex, duration of diabetes, HbA1c adjusted (ß 0.16, p=0.019)) (*Table 5B*).
All analyses were corrected for presence of autoimmune disease and recent infection or hospital admission in a sensitivity analysis. Furthermore the analyses for nephropathy and MDRD were corrected for MAP and the analyses for macrovascular complications, PAD or ABI were corrected for MAP and current smoking. These sensitivity analyses did not change the results (data not shown).

Table 5A SAF levels in patients with and without specific diabetes complications

	No	Yes	p-value
Microvascular			
Neuropathy	2.7 (0.6)	3.1 (0.7)	<0.001
Retinopathy	2.8 (0.6)	2.9 (0.7)	NS
Nephropathy	2.7 (0.6)	3.2 (0.8)	<0.001
Macrovascular	2.7 (0.6)	3.0 (0.7)	<0.001

Table 5B SAF levels in patients with increasing number of complications

	Mean (SD)	N	P versus no complication
Microvascular		309	
0 complication	2.7 (0.6)	150	
1 complication	2.8 (0.5)	107	0.23
2 complications	3.1 (0.8)	42	0.002
3 complications	3.7 (0.9)	10	<0.001

Parameters are expressed as mean (SD)
Neuropathy is defined as a score on the neurothesiometer of ≥ 25 V on the left or the right foot. Retinopathy is defined as any degree of retinopathy (as assessed by fundus photographs). Nephropathy is defined as GFR (MDRD) < 60 ml/min/173m^2. Macrovascular complication is defined as a composite endpoint (MI or CVA or vascular surgery or angioplasty (coronary, carotid, femoral, iliacal, aortal)). P versus no complication: p-values for comparison with SAF levels in patients with 0 complications

Discussion

This cross-sectional observational study comprehensively investigated associations of accumulation of glycation end products (AGEs), measured as SAF with micro- and macrovascular endpoints in type 2 diabetes. Elevated SAF levels were associated with neuropathy, nephropathy, proliferative retinopathy and peripheral artery disease. The association of elevated SAF with worse values on neurothesiometer, MDRD and ABI confirmed these findings. Moreover elevated SAF levels were associated with an increased number of complications. SAF was not associated with macrovascular complications after correction for confounders.

A strong association of SAF and neuropathy was found. Our study showed that SAF levels were not only elevated in patients with neuropathy confirmed by neurothesiometer, but also with values measured by neurothesiometer in a linear fashion in the normal as well as the abnormal range. SAF levels were thus already associated with moderately decreased vibration sensation, even below the threshold of 25 Volt. This is in concordance with prior literature in

which Meerwaldt et al showed that SAF was negatively associated with sensory nerve conduction velocity in a linear fashion in a small study of 24 type 1 and type 2 diabetes patients [20]. Experimental studies in diabetic rat show that AGE accumulation is involved in (progression of) diabetes neuropathy[21, 21, 22], which is in concordance with our findings.

SAF was also strongly associated with nephropathy, which was defined in our study as a decreased GFR. This is in concordance with literature, showing elevated SAF levels in patients with nephropathy[9]. Like values on neurothesiometer; MDRD was also linearly associated with SAF. However, AGEs and AGE precursors are normally cleared by the kidney and decreased clearance might elevate AGE levels[23, 24]. The degree of contribution of diminished clearance is not known. We can therefore not draw any conclusion on cause and consequence in this cross-sectional study.

SAF was associated with macrovascular complications only in crude analyses, which was confirmed by associations of SAF levels with ankle brachial index (ABI) and peripheral artery disease (PAD). The associations with macrovascular complications lost significance after correction for covariates, while association of SAF with PAD remained significant after correction. Furthermore the effect size of associations of SAF with macrovascular complications was comparable with previous literature (HR 1.4 – 1.9)[25]. This suggests that SAF is in fact associated to macrovascular complications, but maybe failed to reach significance due to our sample size.

Our findings are in accordance with our previous review in which we showed a positive association between SAF and one or more diabetes complications, with the exception of retinopathy. The lack of association with retinopathy is in contrast to recent studies showing an association of SAF with retinopathy[10-12] and even associations of skin auto-fluorescence with lens and corneal auto-fluorescence[26]. In our study we did not detect an association of SAF with retinopathy as defined before start of the study (any degree of retinopathy as assessed by fundus photographs). However, SAF was associated with an increased risk of proliferative retinopathy as compared to non-proliferative retinopathy in exploratory analyses. This stresses the importance of definition of endpoints and could contribute to explain the differences in studies described. For instance Gerrits et al specified retinopathy as at least background retinopathy and did not find an association[23], whereas Tanaka et al divided retinopathy in proliferative and non-proliferative and found a higher SAF level in patients with proliferative retinopathy[12]. Altogether, these results indicate that SAF is only associated with proliferative retinopathy.

As shown in our study the association of SAF with complications was not altered by HbA1c. This indicates that SAF is independently associated with outcome. HbA1c is a reflection of hyperglycemia only, while SAF increases with

hyperglycemia, but also oxidative stress. Furthermore HbA1c is more a short-term reflection of metabolic control, whereas SAF is a long-term reflection of glycemic control.

SAF is associated with diabetes complications separately, but also with number of complications. However it is unclear whether SAF is merely a marker of damage or that SAF has a causal role in the (progression of) diabetes complications. Several mechanisms have been proposed by which accumulation of AGEs could result in damage. Accumulation in extracellular matrix could reduce elasticity and lead to arterial stiffness, interaction with the receptor for AGEs or intracellular AGE formation could alter gene expression release cytokines and induce harmful effects[27]. A pathophysiological role for AGEs has been confirmed from experimental studies (in vitro or animal experiments) with AGE-inhibitors and AGE cross-link-breakers[27]. Agents like aminoguanidine, alagebrium and pyridoxamine showed prevention of (progression) of for instance macrovascular complications, neuropathy, retinopathy and nephropathy in animal models[27]. However, this could not be confirmed convincingly in human clinical studies. For example, a study with aminoguanidine in type 1 diabetes patients showed a slower progression of nephropathy and retinopathy in treated patients, but failed to reach the primary endpoint (doubling of creatinine)[28]. Moreover, safety concerns precluded further investigation with this agent. A study with alagebrium showed improvement in pulse pressure, but without an effect on mean blood pressure[29], while a long-term follow-up study did not find an effect of alagebrium on exercise tolerance in patients with systolic dysfunction[30]. In conclusion, these studies do not provide definite proof of a causal relation between AGE accumulation and diabetes complications in humans.

The strengths of this study are its comprehensive assessment of patients characteristics, macro- and microvascular complications and the standardized assessment of these complications at time of inclusion.

This study also had certain limitations. Since it is a cross-sectional study, we can only report associations of SAF with different diabetes complications, but cannot draw conclusions on cause and consequence of these associations. Associations of SAF with different endpoints is influenced by the definition of these endpoints. This could perhaps explain why different studies showed different results, for instance for retinopathy. Nephropathy can also be assessed in different ways. Since many patients (68%) in our cohort used an angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, and preceding micro-albuminuria could not be assessed due to the cross-sectional nature of the study, we chose to define nephropathy as a decreased GFR, which has the limitation that mild nephropathy (only micro-albuminuria) without reduced GFR will be missed. This could underestimate the association of SAF and nephropathy in this study.

In conclusion this cross-sectional observational study showed that accumulation of AGEs, measured as elevated skin autofluorescence in patients with type 2 diabetes was associated with neuropathy, nephropathy, proliferative retinopathy and peripheral artery disease. This was confirmed by continuous associations with values on neurothesiometer, MDRD and ABI. Furthermore elevated SAF was associated with increasing number of microvascular complications. The clinical implication of these associations awaits intervention studies.

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Chapter 8

Advanced Glycation End Products, Measured as Skin Autofluorescence, at Diagnosis in Gestational Diabetes Mellitus Compared with Normal Pregnancy

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Abstract

Background

Advanced glycation end products (AGEs) are tissue proteins that accumulate with age and in diabetes mellitus (DM). AGEs can be measured by the AGE-Reader, which measures skin autofluorescence (SAF). SAF has been suggested as a measure to screen for undiagnosed DM or impaired glucose tolerance. SAF has never been investigated in gestational DM (GDM). Therefore, we compared SAF at diagnosis in GDM patients with normal pregnancy. If SAF is elevated in GDM, future research could focus on the possible use of the AGE-Reader as a screening method for GDM.

Methods

In this monocenter observational study SAF was measured in 60 GDM patients at diagnosis and 44 pregnant women without diabetes.

Results

SAF did not differ between GDM at diagnosis (mean [SD], 1.74 [0.31] arbitrary units) and normal pregnancy (1.76 [0.32] arbitrary units). SAF was lower in white European patients than in patients with other ethnicity.

Conclusions

This first study of tissue AGE accumulation in pregnancy shows no differences in SAF between women with GDM at diagnosis and normal pregnancy. This is most likely due to mild severity and short duration of hyperglycemia in GDM at diagnosis, but it does not exclude potential differences in SAF later in pregnancy. However, the fact that no differences are detected at diagnosis makes it unlikely that the AGE-Reader can be developed as a screening method for GDM in the future. Furthermore, we found that ethnicity should be taken into account when measuring SAF.

Introduction

Advanced glycation end products (AGEs) are modified tissue proteins that can accumulate in different tissues in the body. AGEs accumulate with age, and accelerated accumulation is seen in conditions with glycemic or oxidative stress[1]. The level of AGEs in the skin can easily be measured by the AGE-Reader (DiagnOptics Technologies BV, Groningen, The Netherlands)[2]. This non-invasive, quick method has been validated with AGEs content in skin biopsy specimens[3,4]. Multiple studies have shown that skin AGEs are elevated in patients with type 1 and type 2 diabetes compared with controls without diabetes and that levels of AGEs are associated with development of diabetes complications[5–11]. Moreover, Maynard *et al*.[12] have shown that the AGE-Reader can be used as a screening method in detecting diabetes; in that study undetected DM and impaired glucose tolerance were identified more sensitively by the AGE-Reader compared with hemoglobin A1c (HbA1c) or fasting plasma glucose.

Gestational DM (GDM) is considered to be a mild form of diabetes. Nevertheless, GDM is associated with an increased incidence of maternal and fetal/neonatal complications[13]. Glucose levels are associated with adverse outcome in a linear way without any obvious thresholds above which risks are elevated[14]. Recent intervention trials have shown that treatment of hyperglycemia can improve pregnancy outcome[15,16].

Prior studies found elevated serum AGEs in GDM[17–19], but data on tissue AGEs are lacking. The only study investigating tissue AGEs (through skin autofluorescence [SAF]) in relation to pregnancy showed elevated SAF in recently pre-eclamptic women; however, in that study SAF was measured 6–7 months after delivery and not during pregnancy, and patients in this study had preeclampsia and not diabetes[20].

Thus, if the AGE-Reader can be used as a screening method for undetected diabetes mellitus, this method could be applied in pregnancy as well. No data are available about tissue AGEs during pregnancy in GDM or in normal pregnancies. In this study we measured SAF in patients with GDM and in pregnant women without diabetes. If SAF would be elevated in GDM at diagnosis, future research could focus on the development of the AGE-Reader as a screening method in the detection strategy for GDM.

Patients and Methods

Study design

This single-center observational prospective study was conducted at the outpatient clinic of the Department of Obstetrics of the University Medical Center Utrecht, Utrecht, The Netherlands. In this outpatient clinic, patients are seen by an obstetrician or midwife in a primary-, secondary-, or tertiary-care setting. Patients with GDM are also treated by a diabetes specialist (internal medicine) and diabetes nurse educator. Patients were included from April 2010 until December 2010. The study was approved by the local ethics committee, and all subjects gave written informed consent before measurements.

Patients

The screening and diagnostic strategy in our center is based on the recommendations of the American Diabetes Association. The diagnosis of GDM is based on an abnormal 100-g oral glucose tolerance test (OGTT), which is usually performed between week 24 and 28 of gestation. Although in the current guidelines a 75-g OGTT could be used, in this study we used the 100-g OGTT. Both the 100-g and 75-g tests have been used in The Netherlands, but during the study period only the 100-g test was used. Subjects at increased risk for GDM (based on risk factors) are first screened by a 50-g glucose test (challenge test), which is considered abnormal when the 1-h post-load value is 7.8 mmol/L (140 mg/L) or more. If this challenge test is positive, a diagnostic OGTT is performed. During this OGTT capillary blood glucose levels are measured in the fasting state and at 1, 2, and 3 h after the intake of 100 g of glucose. Patients are diagnosed with GDM if two or more of the cutoff points are met or exceeded; normal values for OGTT are as follows: 0 h <5.3 mmol/L (95 mg/L), 1 h <10.0 mmol/L (180 mg/L), 2 h <8.7 mmol/L (157 mg/L), and 3 h <7.8 mmol/L (140 mg/L)[21]. In case of clinical suspicion of GDM (fetal growth acceleration, large for gestational age, or polyhydramnios), the 100-g OGTT is performed without a prior screening test. GDM patients included in our study all had a positive OGTT. The control group of pregnant women without diabetes can be divided into two subgroups. One group consisted of pregnant women for whom an OGTT or challenge test was performed because of risk factors or clinical suspicion of GDM, but testing was negative. These women were included if an OGTT was performed in which all four glucose values were below the cutoff points or if a challenge test was negative[21]. The other subgroup consisted of pregnant women without any risk factor for GDM for whom an OGTT or challenge test was not performed.

SAF was measured in all subjects during OGTT (mean of four values), or if measurement of SAF during OGTT was not possible because of logistic reasons, SAF was measured after OGTT (within 3 weeks). Because it was unknown which SAF measurement we should take during OGTT, we investigated if SAF changed during OGTT in 37 patients. This group of 37 subjects consisted of 20 GDM patients, 10 patients with only one abnormal value at OGTT, and seven pregnant women without diabetes. No changes in SAF were detected during OGTT (*P* value for time, not significant). Furthermore, changes in SAF did not differ between the groups (*P* value for time ´ group, not significant). Therefore the mean of four SAF values was taken for further analysis. Inclusion criteria were gestational age at OGTT of 20–32 weeks and sufficient knowledge of the Dutch language. Exclusion criteria consisted of pre-existent type 1 or type 2 diabetes, renal failure (glomerular filtration rate <30 mL/min), pre-eclampsia at the time of inclusion or in a previous pregnancy, serious infection or hospital admission during the last 6 months, active autoimmune disease, current use of corticosteroids, smoking, or skin reflectance <6% (if the percentage of reflected light by the skin is less than 6% [usually in patients with dark brown or black skin], then measurement by the AGE-Reader is not possible).
All patients with GDM were treated by a diabetes specialist and diabetes nurse educator following standard protocol consisting of monitoring, diet, and if necessary insulin. Pregnant women without diabetes were treated by their midwife or obstetrician, following standard protocol.

SAF (AGE-Reader)

The level of AGEs in the skin was measured noninvasively with the AGE-Reader, a desktop unit on which the patient positions the volar side of the right lower arm on a light source. The excitation light source is an ultraviolet-A black light tube, with a wavelength between 350 and 420 nm (peak wavelength of 370 nm), which illuminates around 2 cm^2 of the skin. A spectrometer detects the reflected light from the skin in the 420–600 nm range. SAF is calculated as the ratio of the total emission intensity and the total excitation intensity and expressed in arbitrary units (AU)[2,3]. The measurements of SAF are validated against levels of AGEs (pentosidine, carboxymethyllysine, and carboxyethyllysine) in skin biopsy specimens in healthy controls, in patients with diabetes, and in patients on hemodialysis[2,4]. Prior reproducibility studies of repeated measurements in 25 healthy volunteers have shown a mean relative error of 5%[22]. Calculations of within-subject reproducibility from our own results using four consecutive measurements of 37 patients (during OGTT) showed comparable results (coefficient of variation: 4.9%). The AGE-Reader has been validated in patients with skin reflectance ≥6%. A correction is made to the SAF value if the reflectance

is between 6% and 12%. If the reflectance is below 6%, mostly in patients with a dark brown and black skin, measurement with the AGE-Reader is not possible.

Clinical data

At the first visit a questionnaire was completed for baseline characteristics such as age, ethnicity, body mass index (BMI), obstetric history, medical history, medication, and family history. Using standard laboratory techniques, blood was analyzed for HbA1c (reference value, 20–42 mmol/mol [4.0–6.0%]) and fructosamine (reference value, <270 µmol/L) at diagnosis.

Statistics

Results are presented as mean and SD for normally distributed continuous parameters. Differences in baseline characteristics were tested using Student's *t* test for continuous variables and using a χ^2 test for categorical variables. Differences in mean SAF between GDM and normal pregnancy (all pregnant women without diabetes) were tested using Student's *t* test. Differences between the GDM group and both control subgroups were investigated using a one-way analysis of variance. Differences in SAF between GDM and pregnant women without diabetes were adjusted for prespecified factors (age, BMI, time since last meal) and significant differences in baseline characteristics, using a linear regression model. Factors possibly associated with SAF (age, BMI, ethnicity [white European or other], time since last meal, category [GDM or control], HbA1c, fructosamine at diagnosis, and gestational age at SAF measurement) were analyzed using a univariate and multivariate linear regression model. A p value of < 0.05 was considered significant. Changes in SAF during OGTT were investigated using analysis of variance for repeated measurements.
The sample size was calculated before start of the study using a study in recently pre-eclamptic women, which showed a difference in SAF of 0.4 arbitrary units (AU) (SD 0.5)[20]. If the expected AGE accumulation in GDM patients would be comparable with pre-eclampsia, then at least 26 patients per group (α of 0.05 and a power of 80%) to show a 28% difference of SAF level in GDM patients compared with non-GDM were required. Because this study was part of a larger ongoing study investigating association of SAF levels during pregnancy and adverse pregnancy outcome, we calculated a minimum of patients required for this substudy. The actual recruited number of patients in this study is higher (60 GDM vs. 44 control patients), and this sample size is sufficient to detect an even smaller difference in SAF (0.28 AU instead of 0.4 AU).

Results

A total of 153 patients signed informed consent. We included 60 GDM patients and 44 control patients. The control group consisted of 44 pregnant women without diabetes, including 21 women who were confirmed by a negative OGTT, five women who were confirmed by a negative challenge test, and 18 pregnant women without risk factors for GDM. Twenty patients had only one abnormal value and could therefore not be diagnosed with GDM but could also not be considered a control; data from these subjects were only used for analysis of SAF during OGTT. Furthermore, we excluded 29 women who had one or more exclusion criterion: smoking (*n* = 14), weeks of gestation <20 or >32 (*n* = 5), skin reflectance <6% (*n* = 2), withdrew consent (*n* = 2), active autoimmune disease (*n* = 1), current use of corticosteroids (*n* = 1), serious infection or hospital admission during the last 6 months (*n* = 1), and control patients with risk factors, but without confirmatory OGTT (*n* = 3).
Baseline characteristics of GDM and control patients are presented in *Table 1*. BMI was significantly lower in pregnant women without diabetes, compared

Table 1. Baseline characteristics of gestational diabetes mellitus patients and pregnant controls without diabetes

	GDM patients (n = 60)	Pregnant women without diabetes (n = 44)
Age (years)	32.5 (5.0)	33.4 (5.1)
BMI before pregnancy (kg/m^2)	27.6 (6.0) *	25.0 (6.3)
Ethnicity (white European/Moroccan/other)	60%22%18%	82%11%7%
Nulliparous	43%	36%
GDM in previous pregnancy	15%*	0%
Gestational age (weeks) at		
Diagnosis	27.2 (2.6)	—
SAF measurement	27.8 (2.7)*	26.6 (3.0)
HbA1c at diagnosis		
mmol/mol	35 (3.8)	—
%	5.3 (0.34)	—
Fructosamine at diagnosis (µmol/L)	198.6 (16.7)	—
Use of insulin during pregnancy	25%	—
Glucose levels at OGTT (n)	60	21
Fasting glucose (mmol/L) (reference<5.3)	5.6 (0.7) **	4.9 (0.3)
Glucose (mmol/L) after OGTT		
1 h (reference <10.0)	11.4 (1.5)**	8.2 (1.1)
2 h (reference <8.7)	9.6 (1.5)**	7.3 (0.8)
3 h (reference <7.8)	7.6 (2.0)**	6.1 (0.9)

All data are expressed as mean (SD).
Significant difference compared with control: *P < 0.05, **P < 0.001.
BMI = body mass index; GDM = gestational diabetes mellitus; HbA1c = hemoglobin A1c; OGTT = oral glucose tolerance test; SAF = skin autofluorescence.

with GDM. Previous GDM was more common in the GDM group as expected, and glucose levels at OGTT of GDM patients were significantly higher than in controls. Gestational age at SAF measurement was 1 week longer in GDM patients.

SAF at diagnosis did not differ between GDM and pregnant women without diabetes: GDM, SAF = 1.74 AU (0.31); controls, SAF = 1.76 AU (0.32) (*Figure 1*). These results remained unchanged after adjustment of SAF for age, BMI, time since last meal, and gestational age at SAF measurement in a linear model. Exclusion of the outlier in the GDM group (*Figure 1*) did not change these results. No differences were detected if SAF of GDM patients was compared with that of either control groups (confirmed by OGGT and/or challenge test, 1.73 [0.33]; or pregnant women without diabetes without risk factors, 1.81 [0.30]) with one-way analysis of variance ($P = 0.67$).

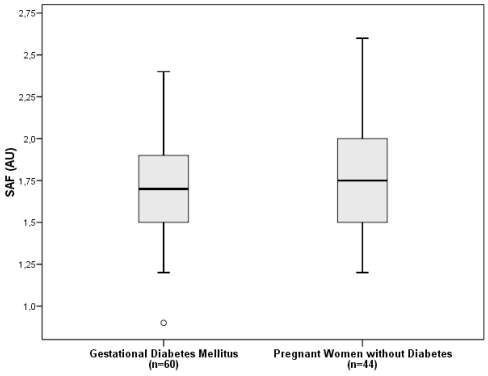

Figure 1. Skin autofluorescence (SAF) values in GDM patients versus pregnant women without diabetes. AU = arbitrary units.

In a linear model containing age, BMI, ethnicity (white European or other), time since last meal, category (GDM or control), HbA1c, fructosamine at diagnosis, and gestational age at SAF measurement, only age and ethnicity were significantly associated with SAF (*Table 2*). With every year a patient gets older, SAF increases 0.02 AU ($P < 0.001$). Patients with ethnicity other than white European had a mean SAF level that was 0.26 higher ($P < 0.001$). Reflectance in other ethnicities than white Europeans was lower. Therefore differences in SAF with ethnicity were corrected for reflection in a multivariate linear model, but this did not change the result. The differences in SAF level between different ethnicities are also shown in *Figure 2*. SAF levels in white Europeans were lower compared with other ethnicity in both patients with GDM and pregnant women without diabetes: GDM group, white Europeans (1.6 AU) versus patients

with another ethnicity (1.9 AU), $P < 0.001$; pregnant women without diabetes, white Europeans (1.7 AU) versus patients with other ethnicity (1.9 AU), $P = 0.07$. Factors such as time since last meal did not have any significant effect on SAF (b = 0.03, $P = 0.43$).

Table 2. Association of skin autofluorescence with different parameters

	Univariate		Multivariate	
	ß-coefficient	P value	ß-coefficient	P value
Age	0.02	0.001	0.02	< 0.001
Ethnicity	0.25	< 0.001	0.26	< 0.001
Time since last food	0.03	0.43		
Category (GDM or control)	0.01	0.74		
Gestational age	–0.01	0.51		
BMI	0.00	0.51		
HbA1c at diagnosis	0.06	0.61		
Fructosamine at diagnosis	0.00	0.56		

BMI = body mass index; GDM = gestational diabetes mellitus; HbA1c = hemoglobin A1c.

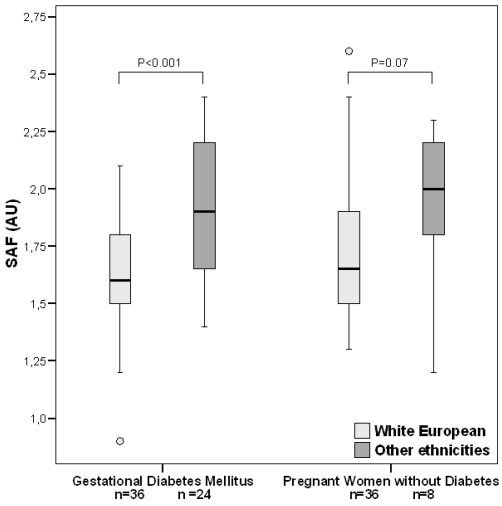

Figure 2. Differences in skin autofluorescence (SAF) between patients of white European origin and patients with other ethnicities. AU = arbitrary units.

Discussion

This is the first study investigating tissue accumulation of AGEs, measured as SAF, in GDM patients compared with pregnant women without diabetes. No accelerated accumulation of AGEs was found in GDM at the time of diagnosis. SAF was lower in white European patients compared with other ethnicities.

Unlike serum AGEs, tissue AGEs have never been measured in pregnant patients before. In contrast to our findings, elevated serum levels of AGEs during pregnancy have been described in GDM patients compared with pregnant women without diabetes[17–19]. However, studies did not always show consistent results: for example, in one study total serum AGE levels in GDM patients were found to be increased, but levels of a single specific AGE (carboxymethyllysine) were actually decreased[17]. In another study serum AGE levels were elevated in GDM patients but not in pregnant patients with pre-existent DM[19], which seems contradictory because hyperglycemia is generally more severe and longer present in pre-existent DM than in GDM. In addition to this, the use of different assays can be a problem in the measurements of serum AGEs[23], and serum AGEs are not necessarily a representation of tissue AGEs, whereas SAF has been validated by actual tissue AGEs in skin biopsy specimens[2,4]. Finally, most of the studies investigating serum AGEs, measured serum AGEs in the third trimester and one also in the second trimester[17–19]. The mean gestational age at measurement in our study was around 27 weeks (second trimester). Therefore we can only conclude from our study that no differences in SAF exist between control subjects without diabetes and GDM patients at diagnosis (second trimester), but we cannot exclude that differences may appear later in pregnancy.

But why did we not find elevated SAF in GDM in our study? Considering that AGE accumulation, measured as SAF, has been found in patients with type 1 and type 2 diabetes in many studies[8–10,24], the absence of a difference between GDM patients and the control population was unexpected. Furthermore, the absolute SAF level we found for GDM patients as well as for pregnant women without diabetes was similar to reference levels for SAF in control subjects as has been published before (SAF levels for control subjects at an age of 30-40 years, 1.73 AU)[25]. There are two possible explanations for the fact that SAF levels were not elevated in GDM patients at the time of diagnosis. First, the hyperglycemia may not have been severe enough to cause accelerated AGE accumulation in these GDM patients at diagnosis. GDM mostly results in mild hyperglycemia (many patients can be treated with diet only). However, accumulation of AGEs has been described before in studies with mild hyperglycemia such as in adequately regulated patients with type 2 diabetes and in patients with only

impaired glucose tolerance[8,12]. Second, the duration of glycemic exposure may have been too short. The exact duration of glycemic exposure in GDM patients at diagnosis is unknown. It is probably no longer than several weeks because GDM patients usually only become insulin resistant and hyperglycemic in the second trimester. Most studies in patients with type 1 and type 2 diabetes have shown that duration of diabetes is directly related to SAF[8–11]. Accumulation of AGEs has always been thought to be a slow process; however, short-term accumulation of AGEs has also been found to occur. SAF was markedly elevated in patients admitted to the intensive care unit (ICU) (patients without diabetes) compared with healthy controls[26]. But the clinical setting of the ICU suggests that AGE accumulation was due to severe non–hyperglycemia-related oxidative stress rather than to hyperglycemia. The absence of evidence of accelerated AGE accumulation at diagnosis in GDM patients does not exclude accelerated accumulation later in pregnancy. Therefore it would be interesting to measure SAF levels during pregnancy in GDM patients. But the fact that SAF is not elevated at the time of diagnosis makes it very unlikely that the AGE-Reader can be developed as a screening method for GDM in the future.

Two factors were significantly associated with SAF: age and ethnicity. The association between age and SAF is well known, and although women in our study did not represent a very wide age range, this association was still found. No prior data exist on SAF in patients with different ethnicity. We found that women with ethnicity other than white European had higher SAF levels. Whether genetic factors or environmental factors (for instance, nutritional habits) contribute to this difference is unknown. In this study the differences in SAF between ethnicity was an unexpected finding for which this study was not designed or powered. However, because differences were very consistent, ethnicity seems to be a factor to take into account when measuring SAF. We did not find an association between HbA1c and SAF. This is in contrast to many studies in patients with type 1 and type 2 diabetes, which have reported a positive association between these two parameters[8–10,27], although an absence of this association has been described too[11]. The fact that we did not find this association could be due to the fact that SAF and HbA1c were measured at diagnosis, and HbA1c levels were usually normal (mean HbA1c in our study was 35 mmol/mol [5.3%]).

We found that AGE levels did not change during OGTT, which confirms prior literature on this topic[28]. There was also no relation between SAF and time since last meal (this was measured in patients within 3 weeks after OGTT), which is in contrast to prior literature in which a 10% elevation of SAF was described following an AGE-rich meal[28,29]. However, in that study the meal contained an abnormally high AGE content, whereas in our study levels were measured in

patients after a normal breakfast. We showed that SAF can be measured at any time during OGTT and at any time after breakfast.

A few limitations of this study have to be addressed. SAF was not measured at the day of OGTT in all patients; some measurements were done days after diagnosis (maximum of 3 weeks) because of logistic reasons. However, separate analyses showed no differences in SAF between patients included on the same day of the diagnosis and patients included within 3 weeks, so it seems that SAF did not change in this short period of time. Furthermore, the exact start of hyperglycemia in GDM is hard to establish, and at a maximum of 3 weeks post-OGTT none of the patients included within 3 weeks had already started insulin treatment.

Serum AGE levels were not investigated in this study. So we cannot compare our results with serum AGE levels. This study was specifically addressing the question if tissue AGEs (measured by SAF) are elevated in GDM at diagnosis.

In conclusion, we showed that SAF (a measure of tissue AGE accumulation) is not elevated in GDM pregnancies at diagnosis compared with pregnancies without diabetes. This could be due to the mild and short duration of hyperglycemia present in this condition at diagnosis and does not exclude potential differences in SAF later in pregnancy. Because we did not find differences in SAF at diagnosis, it is unlikely that the AGE-Reader can be developed as a screening method for GDM in the future, although this study was not specifically designed or powered to investigate this. Furthermore, we found that not only age, but also ethnicity, should be taken into account when measuring SAF.

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Chapter 9

Advanced Glycation End Products, Measured as Skin Autofluorescence, During Normal Pregnancy and Pregnancy Complicated by Diabetes Mellitus

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Abstract

Background

Advanced glycation end products (AGEs) accumulate with age and in diabetes mellitus. AGEs can be measured by the AGE Reader using skin autofluorescence (SAF). SAF is related to chronic diabetes complications. In a previous study we reported that SAF is comparable in patients with gestational diabetes mellitus (GDM) and controls at 27 weeks of gestation. In the current study we investigated SAF at multiple time points during pregnancy in pregnancies complicated by type 1 or type 2 diabetes or GDM and in controls. Furthermore, the relation between SAF levels and adverse pregnancy outcomes was investigated.

Subjects and Methods

In this single-center prospective observational study SAF was measured during pregnancy from 26 gestational weeks onward in 79 GDM patients, 21 patients with pre-existent type 1 or type 2 diabetes and 55 women without diabetes. Adverse pregnancy outcomes were recorded.

Results

SAF decreased slightly but significantly (ß = –0.018) during normal pregnancy but not in pregnancies complicated with hyperglycemia. At the end of pregnancy SAF was higher in patients with pre-existent diabetes (1.91 arbitrary units (AU)) compared with patients with GDM (1.71 AU) or normal pregnancy (1.66 AU) but did not differ between the latter two groups. SAF was not related to adverse pregnancy outcomes.

Conclusions

The decrease in SAF during normal pregnancy could be the result of physiological changes. Because SAF was not related to adverse pregnancy outcomes, it is unlikely that the AGE Reader will be of use in daily clinical practice for GDM patients as a marker for identifying high-risk pregnancy outcomes.

Introduction

Advanced glycation end products (AGEs) are modified tissue proteins that can accumulate in different tissues in the body. AGEs accumulate with age and accelerated accumulation is seen in conditions with glycemic or oxidative stress[1]. The level of AGEs in the skin can easily be measured by the AGE Reader (DiagnOptics Technologies BV, Groningen, The Netherlands)[2]. This noninvasive method (measuring skin autofluorescence [SAF]) has been validated with AGEs content in skin biopsy specimens[3,4]. Multiple studies have shown that skin AGE levels are elevated in patients with type 1 and type 2 diabetes compared with controls without diabetes and that SAF levels are associated with development of diabetes complications[5–12].

In a previous study we reported that there are no differences between SAF levels in gestational diabetes mellitus (GDM) patients and control patients in midpregnancy (27 weeks), which indicates that the AGE Reader is not useful as a screening method for GDM[13]; however, our prior study does not rule out any differences later in pregnancy. Nor does it describe if SAF changes during pregnancy and if differences in changes exist between normal pregnancies and pregnancies complicated by hyperglycemia. Furthermore, literature in nonpregnant diabetes patients shows that AGE levels are strong predictors of future diabetes complications[11,14]. We hypothesized that SAF, measured during or at the end of pregnancy, could predict adverse pregnancy outcomes specific for pregnancies complicated by hyperglycemia. Therefore in this study we investigated whether SAF levels measured during pregnancy are related to adverse pregnancy outcomes. Because no data exist on SAF levels in pregnant patients with pre-existent type 1 or type 2 diabetes, we planned to compare data from GDM and control patients with this group.

Patients and Methods

Study design

This single-center prospective observational study was conducted at the combined outpatient clinic for pregnant women with diabetes of the Departments of Obstetrics and Internal Medicine of the University Medical Center Utrecht, Utrecht, The Netherlands. In this outpatient clinic, pregnant patients are seen by an obstetrician or midwife in a primary-, secondary-, or tertiary-care setting. Patients with type 1 or type 2 diabetes or GDM are also treated by a diabetes specialist (internal medicine) and diabetes nurse educator. Patients were included from April 2010 until December 2011. The study was

approved by the local ethics committee, and all subjects gave written informed consent before measurements.

Patients

For this study patients were included if diagnosed with pre-existent type 1 or type 2 diabetes or with GDM (two or more values above the threshold in a 100-g oral glucose tolerance test [OGTT]). Screening and diagnostic tests for GDM had to be performed between weeks 20 and 32 of pregnancy for patients to be included in the study. The control group of pregnant women without diabetes consisted of two subpopulations. One group consisted of pregnant women in whom an OGTT or glucose challenge test was performed because of maternal risk factors or clinical suspicion of GDM, but in whom testing was negative. These women were included if all glucose values of the OGTT were below the cutoff points or if the challenge test was negative. The other group consisted of pregnant women without any risk factor for GDM, in whom an OGTT or challenge test was not performed.

Exclusion criteria in all three different groups were as follows: renal failure (glomerular filtration rate, <30 mL/min); pre-eclampsia at the time of inclusion or in a previous pregnancy; serious infection or hospital admission during the last 6 months; active autoimmune disease; current use of corticosteroids; smoking; or skin reflectance <6% (because measurement by the AGE Reader is not reliable if the reflectance is <6% [usually in patients with dark brown or black skin]). For the analysis of adverse pregnancy outcome with SAF only patients with a singleton pregnancy were included.

All patients with GDM or type 1 or type 2 diabetes were treated by a diabetes specialist and diabetes nurse educator following standard protocol consisting of glucose monitoring, diet, and if necessary insulin for GDM and insulin treatment for type 1 or type 2 diabetes patients. Pregnant women without diabetes were treated by their midwife or obstetrician, following standard protocol.

SAF (AGE Reader)

The level of AGEs in the skin was measured noninvasively by the AGE Reader. The AGE Reader is a desktop unit on which the patient positions the volar side of the right lower arm on a light source. The excitation light source is an ultraviolet-A black light tube, with a wavelength between 350 and 420 nm (peak wavelength, 370 nm), which illuminates around 2 cm^2 of the skin. A spectrometer detects the reflected light from the skin in the 420–600 nm range. SAF is calculated as the ratio of the total emission intensity and the total excitation intensity and expressed in arbitrary units (AU). The measurements of

SAF were validated against AGE levels (pentosidine, carboxymethyllysine, and carboxyethyllysine) in skin biopsy specimens in healthy controls, in patients with diabetes, and in patients on hemodialysis[2,4]. Prior reproducibility studies of repeated measurements in 25 healthy volunteers have shown a mean relative error (coefficient of variance [COV]) of 5%[15]. Calculations of within-subject reproducibility from our own results, using four consecutive measurements of 37 patients (during OGTT) showed the same COV (4.9%)[13]. The AGE Reader has been validated in patients with skin reflectance ≥6%. A correction is made to the SAF value if the reflectance is between 6% and 12%. If the reflectance is below 6%, measurement with the AGE Reader is not possible. Patients were measured in predefined time windows during pregnancy: Weeks 26–29, Weeks 30–33, Weeks 34–37, and Week >38 and at their first visit postpartum (approximately 8 weeks postpartum).

Clinical data

At the first visit a questionnaire was completed, including baseline characteristics such as age, ethnicity, body mass index, obstetric history, medical history, medication, and family history. Using standard laboratory techniques, blood was analyzed for glycated hemoglobin (HbA1c) (reference value, 20–42 mmol/mol [4.0–6.0%]) at diagnosis. Data for adverse pregnancy outcomes were retrieved from patients' records. Primary end point was large for gestational age (LGA), and secondary end points included pre-eclampsia, preterm delivery, cesarean section, fetal death (>20 weeks), hypoglycemia, neonatal jaundice, and birth trauma. LGA was defined as >90th percentile, corrected for gestational age, sex, and parity, according to the new Dutch reference curves[16]. Preterm delivery was defined as delivery before 37 completed weeks, neonatal hypoglycemia was defined as glucose <2.0 mmol/L in the first 24 h or need for glucose infusion, and neonatal jaundice was defined as requirement of phototherapy and shoulder dystocia as reported in the patient record. Finally, a composite end point was defined including one or more of the abovementioned adverse pregnancy outcomes.

Statistics

Results are presented as mean and SD values. Differences in baseline characteristics were tested using a Student's *t* test for continuous variables and using a χ^2 test for categorical variables. A value of $P < 0.05$ was considered significant.

First, whether SAF changes during pregnancy differed among the three groups was analyzed using analysis of variance for repeated measurements with time

point during pregnancy, pregnancy group, and the interaction between time and pregnancy group in the model. In case the interaction term was significant, changes over time among the three groups were considered different. Patients with three or more values were included in this analysis and missing values were imputed. Imputation was carried out as follows: when the first or the fourth value was missing, the data were copied from the second or the third value, respectively. When the second or the third measurement was missing, the average of the first and third or of the second and fourth was taken, respectively. To assess reliability of this method of imputation, an alternative method of imputation was used as well, using the last observation carried forward.

Next, the relation between pregnancy week and SAF was assessed in all three groups separately using linear regression. Finally, differences among SAF in GDM, control, and pre-existent DM patients at a certain time point (cross-sectional) during pregnancy were analyzed using one-way analysis of variance with Bonferroni's correction. Differences between SAF before delivery and SAF postpartum within one group were analyzed with a paired *t* test. All differences found above were adjusted for prespecified factors (age and ethnicity) using a linear regression mode because these factors were identified as the most important confounders in a previous study[13].

A logistic regression model was used to investigate the relation of SAF with adverse pregnancy outcomes. This analysis of relation of SAF with adverse pregnancy outcomes was carried out in all three groups together (GDM, control, and pre-existent DM), with pre-existent DM (yes/no) as a factor in the logistic regression analysis. This analysis was performed for the primary end point (LGA) age and for the other two end points with the most cases (cesarean section and the composite end point).

The sample size necessary to investigate any differences between SAF in different groups was calculated based on a study in recently pre-eclamptic women, which showed a difference in SAF of 0.4 AU (SD 0.5)[17]. If the expected AGE accumulation in GDM patients or patients with pre-existent DM would be comparable to that in patients with pre-eclampsia, then at least 26 patients per group (α of 0.05 and a power of 80%) were required.

The sample size necessary to investigate a relation between SAF and adverse pregnancy outcomes was also calculated using previous studies showing odds ratios ranging from 3 to 4 for adverse pregnancy outcome with hyperglycemia during pregnancy[18,19]. Historical (authors' unpublished) data from our own clinic were used to estimate the percentage of patients who would suffer adverse pregnancy outcomes, such as LGA (26%). Assuming that the risk of adverse pregnancy outcomes is of the same magnitude with increased SAF, a sample size of 100 GDM patients would be sufficient to detect a relative risk of

2.15 for the primary end point (LGA) (assuming that LGA would occur in 26% of the patients, power 80%, α = 0.05). And, 2.15 is well below the odds ratio (3–4) previously found.

Results

In total, 197 patients signed informed consent. We included 79 GDM patients and 21 patients with pre-existent DM (13 with type 1 diabetes and eight with type 2 diabetes). The control group consisted of 55 pregnant women without diabetes, including 38 women who had a negative OGTT or negative challenge test and 17 pregnant women without risk factors for GDM. We excluded 42 women because of smoking (*n* = 17), gestational age <20 or >32 weeks (*n* = 7), skin reflectance <6% (*n* = 3), withdrawal of consent (*n* = 2), active autoimmune disease (*n* = 1), current use of corticosteroids (*n* = 1), serious infection or hospital admission during the last 6 months (*n* = 1), pre-eclampsia (*n* = 3), control patients with risk factors in whom no challenge test or OGTT was performed (*n* = 2), or incorrect interpretation of OGTT (*n* = 5). During the study 24 patients were lost to follow-up (six GDM patients, 15 control patients and three pre-existent DM patients).

Baseline characteristics of GDM patients, patients with pre-existent DM, and control patients are presented in *Table 1*. Body mass index was significantly lower in pregnant women without diabetes, compared with patients with GDM or pre-existent DM. HbA1c in the second trimester was higher in patients with pre-existent DM compared with patients with GDM. HbA1c was not measured in control patients.

Table 1. Baseline characteristics of the three groups

	Pregnant women without diabetes (n = 55)	GDM patients (n = 79)	Pre-existent DM patients (n = 21)
Age (years)	33.1 (5.2)	32.7 (4.9)	32.9 (4.7)
BMI before pregnancy (kg/m^2)	25.0 (6.0)	28.2 (6.4)[a]	29.5 (8.4)[a]
Ethnicity (white European/Moroccan/other)	84%/9%/7%	63%/19%/18%	67%/24%/9%
Nulliparous	46%	46%	48%
HbA1c in 2nd trimester (mmol/mol)	—	34.0 (3.8)	43.2 (8.1)[b]
Use of insulin during pregnancy		27%	100%

Data are mean (SD) values or percentages.
[a] Significantly elevated compared with pregnant women without diabetes.
[b] Significantly elevated compared with gestational diabetes mellitus (GDM) patients.
BMI = body mass index; DM = diabetes mellitus; HbA1c = glycated hemoglobin.

The changes of SAF levels during pregnancy in different groups are shown in *Figure 1*. Direction and course of SAF changes during pregnancy were comparable between the groups (time x group not significant). We did find a significant difference in mean SAF between the groups (group; $P = 0.045$) (mean SAF of pre-existent DM during pregnancy was elevated compared with the other groups). Changes of SAF during pregnancy were also significant (time; $P < 0.05$), showing an overall decrease of SAF over time during pregnancy. Results were comparable when using two different ways of imputation of data. SAF

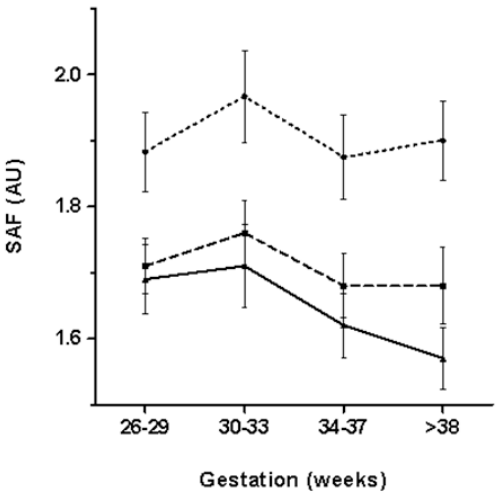

Figure 1. Skin autofluorescence (SAF) levels during pregnancy in patients with pre-existent diabetes mellitus (DM), gestational diabetes mellitus (GDM), and control patients. AU = arbitrary units.

decreased over time in all three groups together with a significant effect of time in the repeated-measurements analysis. Therefore weeks of gestation as a factor was investigated in a linear model in the three different patient groups separately (control, GDM, and pre-existent DM) adjusting for age and ethnicity (white European or other) (*Table 2*). This showed that the decrease in SAF during pregnancy was significant in control patients only, showing a decrease of 11% during pregnancy.

Differences in SAF were also analyzed cross-sectionally among the groups at 26–29 weeks and later in pregnancy. SAF was elevated in patients with pre-existent DM, compared with pregnant women without diabetes in weeks 26–29 (pre-existent DM, 1.93 AU [0.21]; controls, 1.75 AU [0.33]), but this difference was not significant. A significant difference was shown later in pregnancy (weeks 34–37 [$P = 0.025$] or week >38 [$P = 0.026$]) (pre-existent DM, 1.91 [0.24]; controls, 1.66 [0.28]; $P = 0.025$ [weeks 34–37]). These results did not change when corrected

for age and ethnicity in a linear regression model. No significant differences were seen between GDM and control patients. As can be seen from *Figure 2*, SAF increased significantly again 8 weeks postpartum in the control patients: SAF before delivery, 1.59 (0.20); post-partum, 1.72 (0.21) ($P < 0.001$).

Table 2. Linear regression analysis of factors associated with skin autofluorescence during pregnancy in three study groups

	Univariate					
	Pregnant women without diabetes		**GDM patients**		**Preexistent DM patients**	
	β	CI	β	CI	β	CI
Duration of pregnancy (weeks)	-0.018	–0.029 to –0.006		NS		NS
Age (years)	0.020	0.011–0.030	0.025	0.016–0.035		NS
Ethnicity (white European or other)	0.36	0.23–0.50	0.27	0.17–0.36	0.14	0.02–0.27

	Multivariate					
	Pregnant women without diabetes		**GDM patients**		**Preexistent DM patients**	
	β	CI	β	CI	β	CI
Duration of pregnancy (weeks)	–0.018	–0.028 to –0.008		NS		NS
Age (years)	0.018	0.010 – 0.026	0.029	0.020–0.038	0.013	0.000–0.026
Ethnicity (white European or other)	0.35	0.23 – 0.47	0.30	0.21–0.39	0.20	0.06–0.33

Data are β values and 95% confidence intervals (CIs). Statistically significant findings are indicated in bold type. DM = diabetes mellitus; GDM = gestational diabetes mellitus; NS = not significant.

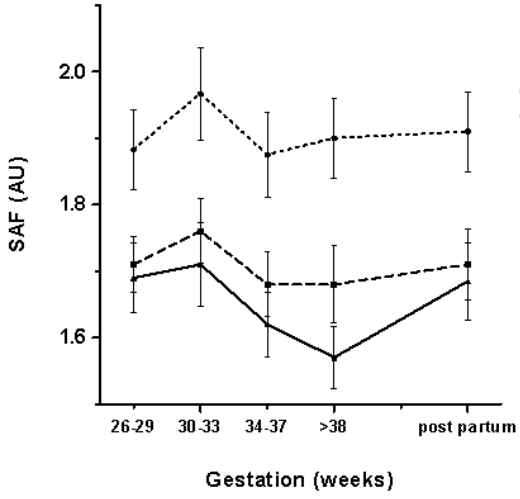

Figure 2. Skin autofluorescence (SAF) levels during pregnancy and post-partum in patients with pre-existent diabetes mellitus (DM), gestational diabetes mellitus (GDM), and control patients. AU = arbitrary units.

The incidence of adverse pregnancy outcomes was lower than expected (*Table 3*). Complication rates in GDM patients and control patients were comparable, but patients with pre-existent DM had significantly more complications (composite end point) and more LGA neonates. SAF was not associated with any of the adverse outcomes or the composite end point, but HbA1c and diagnosis of pre-existent DM were associated with an increased risk of adverse pregnancy outcome (*Table 4*). Pre-existent DM was, as expected, the strongest factor. However, HbA1c was also independently associated with adverse pregnancy outcome when investigated in the GDM group only.

Table 3. Adverse pregnancy outcomes in three study groups

	Pregnant women without diabetes	**GDM patients**	**Pre-existent DM patients**
Any complication	40%	42%	79%[a]
Large for gestational age	5%	7%	37%[a]
Cesarean section	29%	30%	53%

Data are percentages.
[a] Significant difference compared with the other groups.
DM = diabetes mellitus; GDM = gestational diabetes mellitus.

Table 4. Relation of skin autofluorescence with adverse pregnancy outcomes, analyzed by univariate logistic regression

	Any complication	Large for gestational age	Cesarean section
SAF	1.6 (0.56–4.7)	1.3 (0.31–1.7)	1.5 (0.55–4.3)
Age	0.99 (0.92–1.1)	0.96 (0.86–1.1)	1.0 (0.93–1.1)
BMI	**1.1 (1.0–1.2)**	1.0 (0.92–1.1)	1.0 (0.99–1.1)
HbA1c	**1.2 (1.1–1.3)**	**1.2 (1.1–1.3)**	**1.1 (1.0–1.2)**
Pre-existent DM (yes/no)	**5.3 (1.7–17)**	**8.6 (2.6–28.7)**	2.6 (0.99–7.1)

Data are odds ratios (confidence interval). Significant odds ratios are indicated in bold type.
BMI = body mass index; DM =diabetes mellitus; HbA1c = glycated hemoglobin; SAF = skin autofluorescence.

Discussion

This is the first study investigating tissue accumulation of AGEs, measured as SAF during pregnancy in GDM patients, in pregnant women without diabetes, and in patients with pre-existent DM. SAF slightly, but significantly, decreased during pregnancy in control patients but not in pregnancies complicated by

hyperglycemia. SAF was elevated in patients with pre-existent DM compared with control and GDM patients. No associations of SAF levels with adverse pregnancy outcomes were found.

Differences in SAF between pre-existent DM and GDM and control patients were more pronounced at the end of pregnancy than in midpregnancy. This was merely due to a decrease in SAF in control patients because this decrease was not seen in hyperglycemia-complicated pregnancies. It is unknown which factors could contribute to the decrease in SAF we found. Several physiological changes occur during pregnancy, such as increased glomerular filtration rate, peripheral vasodilation, and increased cardiac output[20]. Changes in renal physiology (increased glomerular filtration during pregnancy) could contribute to a higher clearance of AGEs because circulating AGEs are cleared by the kidney[21]. A decrease in SAF seems contradictory to the irreversible nature of AGE formation, but the lifetime of AGEs is determined not only by AGEs itself, but also by the turnover of the AGE-modified protein[22]. An acute rise, followed by a decrease, in SAF has been described before in patients after myocardial infarction, but never in a physiological state such as pregnancy[23]. Another possibility is that cardiovascular changes, such as peripheral vasodilation, influence SAF measurement itself. SAF has been reported to be decreased when vasodilation occurs (induced thermally or pharmacologically), possibly because of absorbent properties of hemoglobin[24]. However, this is less likely to be a significant factor in our study because vasodilation is usually more prominent earlier in pregnancy and was not induced externally and probably less profound. Apparently hyperglycemia (in GDM and pre-existent DM) prevents the decrease in SAF.

In a prior study we found that SAF was comparable in GDM at diagnosis and control patients in the same week of pregnancy[13]. The relatively mild and short hyperglycemia could be the reason that SAF levels are not yet elevated in GDM pregnancies at diagnosis. In the present study we found that a difference in SAF between GDM and control patients later in pregnancy did not develop either, indicating that even with longer exposure to hyperglycemia, the hyperglycemia experienced in GDM is too mild to elevate SAF. We did see elevated SAF in patients with pre-existent DM, in whom hyperglycemia is more explicit and of longer duration.

This study did not show any relation of SAF levels with adverse pregnancy outcomes. We did find the expected association between HbA1c and adverse pregnancy outcomes in patients with pre-existent DM or GDM (HbA1c was not measured in control patients). Therefore HbA1c as a long-term parameter of glycation remains the parameter of interest.

A few methodological issues of the study have to be addressed. First, the sample size necessary to investigate differences in SAF between different groups was calculated at 26 patients per group. For the group of patients with pre-existent DM this sample size was not met because of logistic reasons. However, because the other two groups were larger than 26 patients, the 21 patients in this group is sufficient. But for the analysis of relation of SAF with adverse pregnancy outcomes, the group of pre-existent DM is rather small to base firm conclusions on. For GDM patients the sample size necessary to investigate a relation between SAF and adverse pregnancy outcomes was estimated at 100. Because of logistic reasons 79 instead of 100 GDM patients could be included. With this sample size we did not find any relation between SAF and adverse pregnancy outcome, but of course very modest associations could have been missed.

Second, in this study without interference with usual care we had a relative high amount of loss to follow-up, mainly in the control group. This could be due to the fact that patients in the control group sometimes returned to the referring clinic or midwife after a negative OGTT. Therefore these patients could not be followed up in this study. However, we do not expect this loss to follow-up to have influenced our results because an analysis of both groups (lost to follow-up and patients followed during the whole study) did not show any statistical differences.

Third, results of SAF in pregnant women have to be interpreted with caution because it is a new group of patients to be explored. The AGE Reader only measures fluorescent AGEs, and some other fluorescent compounds in the skin could be confounders in this measurement[15]. However, the AGE Reader has been validated with skin biopsy specimens (including fluorescent and nonfluorescent AGEs) in healthy controls, in patients with diabetes, and in patients on hemodialysis[2,4], and there is no a priori reason to assume that the technique would not be valid in pregnant women.

In conclusion: SAF decreases during normal pregnancy and increases again after delivery. This decrease appears to be overruled by hyperglycemia. SAF is higher in patients with pre-existent DM but is comparable in GDM and control patients. SAF was not associated with adverse pregnancy outcome. It is therefore unlikely that the AGE Reader will be of use in daily clinical practice for GDM patients because data from our previous study[13] do not suggest that it can be used to screen for undetected GDM, nor do data from the present study show that it can be used to identify patients at risk for adverse pregnancy outcomes.

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Chapter 10

General Discussion

In this thesis we investigated glycemic variability and AGEs as alternative parameters of glycemic control. HbA1c is a measure of the mean glucose levels over the past few months. Although HbA1c lowering directly results in reduced diabetes complication risk, HbA1c does not provide all information about glycemic control. HbA1c does not provide information about actual glucose levels, glucose peaks and nadirs, whereas glycemic variability does. Furthermore HbA1c does not explain metabolic memory, whereas accumulation of advanced glycation end products (AGEs) may serve as a more long-term reflection of hyperglycemia and tissue glycation.

Glycemic variability

Diabetes is not only characterized by hyperglycemia, but also fluctuating glucose levels. While in normal subjects glucose levels are kept in a very small range, glycemic variation is much larger in diabetes patients[1]. If the body puts so much energy in keeping glucose levels in a very stable range in the physiological situation, one could imagine that variability of glucose levels is harmful for diabetes patients. Glycemic variability has indeed been suggested to be an important risk factor for development of diabetes complications, and fluctuating glucose levels have been shown to be more deleterious than chronic hyperglycemia alone[2, 3]. However, controversy about the topic remains since studies investigating the association between variability and diabetes organ complications showed inconsistent results[4-10].

A possible pitfall in the studies investigating variability and complication risk could be the temporary nature of the measurement of glycemic variability. This is measured at a certain (short) time interval, whereas chronic diabetes complications could arise over many years. Relating glycemic variability to risk of diabetes complications assumes that variability is a constant phenomenon in one patient, whereas one could imagine that it changes when therapeutic regimen is altered or with increasing duration of disease.

We showed in chapter 2 that glycemic variability is indeed not constant, at least not in type 2 diabetes patients. Whereas in subjects without diabetes glycemic variability (measured as SD of blood glucose levels) is approximately 1 mmol/L[11], glycemic variability in type 1 diabetes patients is approximately 3.6 mmol/L, and in type 2 diabetes it depends on type of therapy and progression of disease. Glycemic variability in patients with type 2 diabetes on intensive insulin scheme was approximately 2.7 mmol/L, but it increases with duration of insulin treatment. Glycemic variability of patients with type 2 diabetes became as high as type 1 diabetes patients (SD 3.6 mmol/L) after approximately 20 years of insulin therapy. With progressive disease in type 2 diabetes, beta-cell mass and endogenous

insulin decrease, which can be followed by alpha-cell dysfunction and larger variability. The difference in variability and the progression in type 2 diabetes patients to a glycemic variability of type 1 diabetes patients could be explained by the different nature of the cause and the progression rate of the disease. This emphasizes that large glycemic variability is not only present in type 1 diabetes, but also an important feature of (longstanding) type 2 diabetes requiring insulin therapy.

Apart from the discussion of the possible role of glycemic variability in (progression of) diabetes complications, we investigated in chapter 3 whether a fluctuating glucose level on its own is bothersome for a patient by relating it to quality of life. In the outpatient clinic, patients indeed frequently complain about variable glucose values. A high degree of glycemic variability can conceivably have a negative impact on quality of life since it leads to more problems with the manageability and control of the disease because of large or unexpected swings in plasma glucose levels. This would especially be of interest in patients with diabetes since these patients already have a decreased quality of life, especially when diabetes complications and/ or hypoglycemia are present[12-15]. If higher glycemic variability decreases quality of life, this would emphasize it as a clinical problem. Two prior cross-sectional studies already showed an association between lower glycemic variability and better health-related quality of life in patients with type 2 diabetes[16, 17]. However both studies in type 2 diabetes investigated a smaller sample of patients and none corrected for HbA1c. We showed that the association was present when quality of life was measured by the disease specific questionnaire Problem Areas in Diabetes Scale (PAID) and mainly in the domains concerned with diabetes-related emotional problems and treatment-related problems. However, the association was only present in type 2 diabetes patients requiring insulin therapy and when corrected for HbA1c it attenuated to non-significant. Although the association between glycemic variability and quality of life may thus not be independent of HbA1c, glycemic variability still has a bearing on daily clinical practice. For example, lowering HbA1c as a treatment goal will usually lead to more hypoglycemia. More hypoglycemia in turn could result in reactive hyperglycemia and increased variability. A dual treatment goal of lowering HbA1c together with lowering the risk of hypoglycemia and glycemic variability seems therefore appropriate in the individual patient. In general, this underlines the importance of a broad scope of relevant end-points in clinical studies in diabetes, including glycemic variability and quality of life.

The temporary nature of the assessment of glycemic variability is an important issue and in future studies glycemic variability could be assessed on multiple occasions. If future studies are performed with glycemic variability these would

also benefit from comparability; that is, using one parameter to measure variability. While HbA1c is a very straightforward measurement, which has been validated all over the world, much more discussion exists about what glycemic variability is and how it should be measured. More than 20 parameters have been described to measure glycemic variability[18]. Rodbard et al. described a strong correlation between standard deviation (SD) and other parameters of glycemic variability in diabetes patients with adequate control. We confirmed these findings in chapter 2 using continuous glucose monitoring in inadequately controlled patients, in which correlations were comparable in strength. We took into account the fact that glucose levels measured by continuous glucose monitoring are not always entirely normally distributed and that calculating SD from a not-normal distribution is in fact mathematically incorrect. However, SD is an easy to measure -and easy to understand parameter. And other parameters also make assumptions that are random and not necessarily correct, like the mean amplitude of glycemic excursions (MAGE), which only takes peaks or nadirs > 1 SD into account, or the continuous overall net glycemic action ($CONGA_n$), which uses SD of summated differences between glucose levels during a certain time period of a randomly n hours apart. Therefore, we propose SD as the parameter of choice.

The second issue in measuring glycemic variability is the best data set to derive this from. What is the maximum of time between consecutive glucose measurements and what is the minimum amount of time in which continuous glucose monitoring has to be performed? Neylon et al suggest that data sets to calculate glycemic variability should at least have a duration of 12 days, since variability calculated from such a data set best approximates variability calculated during 90 days[19]. However, they use 90 days as a gold standard, whereas again no consensus about a gold standard exists. What is interesting in this observation, is that it emphasizes the temporary nature of continuous glucose monitoring. We think that a minimum of days that patients have to wear continuous glucose monitoring to calculate variability cannot be given. However, we suggest at least more than 24 hours to incorporate one day and night.

In conclusion, studies with glycemic variability would benefit from comparability of parameters of this entity, we suggest the parameter of choice to be SD, calculated from glucose levels from continuous glucose measurement of at least 24 hours. Glycemic variability is not a static entity, and changes with progression of disease in type 2 diabetes. Since lowering HbA1c in a patient with continuous swings between high and low blood glucose levels would inevitably result in more (undesirable) hypoglycemia and glycemic variability is directly associated with quality of life, specific interventions to lower variability

are worthwhile to investigate. Such a study could comprehend for instance an intervention study, aiming to decrease variability in patients with known large variability (for instance long-standing type 2 diabetes on intensive insulin scheme) and should encompass, not only the usual biochemical and clinical end points, but also investigate glycemic variability and quality of life as additional end points.

Potential interventions to improve glycemic variability

Intervention in the incretin pathway could theoretically be an important tool to improve glycemic variability. Glycemic variability is determined by hyperglycemic and hypoglycemic excursions. DPP4-inihbitors have effects on alpha and beta cell function[20]. An increase in the first insulin response after a meal (beta cell function) together with a decrease in postprandial glucagon (alpha cell function) could reduce hyperglycemic excursions, while an increase in glucagon response following a hypoglycemia could reduce a hypoglycemic excursion[20].

In chapter 5 we describe a small randomized study investigating if the addition of vildagliptin to start of insulin treatment in patients with type 2 diabetes could reduce glycemic variability and if this could reduce required units of insulin for glycemic control (main end-point). We hypothesized that a DPP4-inhibitor could lead to less exogenous insulin requirements in this group of patients with residual beta-cell function. Lower insulin use could reduce side effects of insulin treatment such as hypoglycemia and weight gain[21] and lower peripheral hyperinsulinemia, thought to have atherogenic and mitogenic effects[22]. Furthermore, personal clinical experience shows that patients are not only reluctant to start insulin therapy, but are also reluctant to increase insulin dose. Therefore smaller insulin doses could lead to better patient acceptance and compliance. Smaller doses of insulin can also be more convenient to patients since large doses can be a practical problem to inject. Our study eventually failed to reach the calculated sample size due to insolvable recruitment problems. From our study we can therefore not provide an answer if a DPP4-inhibitor reduces required insulin dose or glycemic variability.

Another way to reduce glycemic variability could be by extensive glucose monitoring, enabling patients to react to minor glucose changes, and in doing so keeping variability as low as possible. The real-time continuous glucose monitoring is the most useful device for this purpose since it can be used continuously to improve glycemic control. Alarms can be set to alert the patient at low or high glucose levels or changes in levels and patients can intervene immediately. Multiple studies have evaluated (real-time) continuous glucose

monitoring in the treatment of diabetes. We reviewed these studies using real-time continuous glucose monitoring in chapter 4. All studies investigated effects on HbA1c and most studies showed a larger reduction in HbA1c with real-time continuous glucose monitoring than in controls. Only three studies investigated the effects on variability[23-25], and only two did show a reduction in glycemic variability[24, 25]. This was confirmed by a later trial showing a decrease in glycemic variability with sensor-augmented pump therapy in type 1 diabetes patients[26].

An important question is in which patients continuous glucose monitoring would be most beneficial. Apart from being expensive, this is a laborious, demanding and time-consuming technique for the patient and the diabetes team alike. It is evident that in order to expect effect of real-time continuous glucose monitoring a patient should wear the device for a minimum amount of time and hence compliance is very important. The effort it takes for a patient to wear a real-time continuous glucose monitoring and to be overloaded with glucose levels (many of them out of range) and alarms should also be taken into account. Real-time continuous glucose monitoring could be especially interesting in patients with recurrent or severe hypoglycemia or large variability. But most studies in our review excluded patients with severe hypoglycemia from entering the study. Recent reports also describe that a treatment algorithm should be provided to the patients using this device and that this could positively influence not only glycemia, but also quality of life[27]. This algorithm should be provided as soon as patients start with real-time continuous glucose monitoring since 16 weeks postponement of the algorithm did not show improvement because patients provided at a later moment with the algorithm might already have their own responses to and expectations of the device[27].

In conclusion, both addition of a DPP4-inhibitor as well as real-time continuous glucose monitoring have the theoretical potential to reduce glycemic variability, and DPP4-inhibitors also could have the potential to reduce required insulin dose. However, our intervention trial with vildagliptin was too small to base conclusions on. Therefore this trial should be considered only as a small pilot study, which can be used to base a power calculation for a larger intervention trial on. Real-time continuous glucose monitoring may have an effect on glycemic variability although specific studies preferably including patients with high variability and with the specific aim to reduce this are needed.

Advanced glycation end products

Apart from the fact that HbA1c is just a reflection of the mean glucose and does not provide information about actual glucose levels as described above, HbA1c is also only a reflection of the glucose levels over the past months, whereas measurement of accumulation of advanced glycation end products (AGEs) can provide information about hyperglycemia for a much longer period of time. The difference between the two parameters is determined by the tissue that is glycated and the turn-over of the different tissues. For HbA1c this is the hemoglobin molecule, with a lifespan of approximately 4 months. While for accumulation of advanced glycation end products measured by the AGE-reader this is skin collagen, which has a half-life of approximately 15 years[28].
In this thesis we evaluated the use of measurement of accumulation of advanced glycation end products, measured as skin autofluorescence (SAF) by the AGE-reader.
We evaluated if the AGE-reader could be used to detect patients with gestational diabetes mellitus in chapter 8 and 9. In contrast to the extensively investigated associations of SAF with diabetes complications in patients with type 1 and type 2 diabetes, the AGE-reader was never investigated in gestational diabetes. Therefore, we first investigated if SAF was elevated in gestational diabetes at all. This is an absolute prerequisite for a measurement to be considered for use as a screening test. However, such an elevation was not found. This was unexpected since we hypothesized that patients that develop gestational diabetes are metabolically different from patients who do not. Gestational diabetes is an important risk factor for developing type 2 diabetes at advanced age, these patients apparently have a decreased beta cell reserve, which cannot meet requirements anymore during insulin resistance caused by pregnancy. But apparently in gestational diabetes patients the elevation of glucose levels was too small and too short to cause AGE accumulation, measureable with the AGE-reader.
In contrast, SAF was higher in pregnant patients with pre-existent diabetes (type 1 or type 2 diabetes), in whom glucose levels are also elevated before pregnancy. Moreover, we unexpectedly found in our study that SAF slightly decreased in normal pregnancy in contrast to pregnancy complicated by diabetes. This is in contrast to the fact that skin collagen has a half-life of approximately 15 years. Local factors or edema could have played a role. Another possibility might be a quicker turn-over of the skin collagen. However, a thorough explanation can only be speculated on.

Apart from the use of the AGE-reader in gestational diabetes we also aimed to review all existing evidence about the AGE-reader and diabetes complications in patients with type 1 or type 2 diabetes in chapter 6. We showed that many studies (cross-sectional as well as prospective) associated elevated AGE accumulation with diabetes complications. However most studies used different definitions of endpoints (diabetes complications). Definition of endpoints is an important issue, since it directly influences results. In chapter 7 we performed an observational study in our own large cohort of type 2 diabetes patients investigating the association of SAF measured by the AGE-reader and all different diabetes complications defined in a dichotomous and in a continuous way in one study. We showed that SAF was associated with neuropathy and nephropathy independent from HbA1c and that association with retinopathy was dependent on the definition of retinopathy (any retinopathy versus proliferative retinopathy). The independent association of macrovascular complications or ankle brachial index could not be confirmed in our study, while the association with peripheral artery disease could.

The question remains whether measurement of SAF has additive value over HbA1c alone. Accumulation of AGEs could result in damage by accumulation in the extracellular matrix, reducing elasticity and leading to arterial stiffness. Interaction with the receptor for AGEs or intracellular AGE formation could alter gene expression release cytokines and induce harmful effects[29].

A pathophysiological role for AGEs has indeed been confirmed from experimental studies (in vitro or animal experiments) with AGE-inhibitors and AGE cross-link-breakers[29]. However, this could not be confirmed convincingly or safely in clinical studies[30]. The question is if treatment goals should be on HbA1c or on SAF reduction. Perhaps it is the reflection of the same entity (a measure of elevated blood glucose levels), but the period which it reflects is different. AGE accumulation (SAF) can detect patients with evidence of long-term glycemic damage and is therefore a better reflection of "metabolic memory" (long-term detrimental effect of hyperglycemia even after improvement of glycemic control) than HbA1c.

In conclusion, the AGE-reader cannot be used to detect gestational diabetes. In type 1 and type 2 diabetes elevated SAF levels are associated with all different diabetes complications, although definition of end points can influence these associations.

Overall conclusion and future perspectives

Glycemic control is a broader concept than HbA1c alone. Individualizing the biochemical target level of HbA1c (not < 53 mmol/mol (<7%) in every patient)

has become common practice. However, alternative potential treatment goals (other than HbA1c) should be individualized too. It depends on the individual patient whether simply a better HbA1c should be achieved or whether other treatment goals like lowering glycemic variability, improving quality of life, decreasing units of insulin or a combination of these goals should be accomplished.

As an example, in a patient with a high HbA1c and continuous swings between high and low blood glucose levels, a sole treatment goal of lowering HbA1c would inevitably result in more (undesirable) hypoglycemia and therefore intuitively decreasing variability is the most obvious initial treatment goal in such a particular patient. Studies investigating glycemic variability would benefit from comparability of parameters of this entity, we suggest the parameter of choice to be SD. Glycemic variability is not only a problem in patients with type 1 diabetes, but also increases with long-standing insulin therapy in type 2 diabetes. Glycemic variability is associated with worse quality of life in type 2 diabetes patients, albeit only in insulin-treated patients. Treating glycemic variability could thus theoretically help to improve quality of life in such patients.

Real-time continuous glucose monitoring as well as addition of a DPP4-inhibitor to existing insulin regimen have the theoretical potential to reduce glycemic variability, and DPP4-inhibitors also could have the potential to reduce required insulin dose. However, our intervention trial with vildagliptin was too small to base conclusions on. A larger trial could also focus on adding a DPP4-inhibitor to type 2 diabetes patients already on intensive insulin scheme and investigate whether this has the potential to reduce variability and insulin dose.

Off-line continuous glucose monitoring can be used as an educational tool, to recognize specific patterns and discuss these with the patient. Real-time continuous glucose monitoring can also be used for these purposes, but has the ability to allow direct treatment decisions too. Real-time continuous glucose monitoring may have an effect on glycemic variability, rendering specific studies preferably including patients with high variability and with the specific aim to reduce this necessary. Future research could focus on prospective follow-up studies in type 2 diabetes on intensive insulin treatment, measuring glycemic variability and quality of life on multiple occasions. This would especially be relevant after interventions to reduce glycemic variability (real-time continuous glucose monitoring or DPP4-inhibitor) to investigate whether an improvement in glycemic variability also improves quality of life.

Whether measurement of accumulation of AGEs has a place in daily clinical practice is questionable. Currently, these measurement are mainly useful for research purposes. If data on the pathophysiological role of AGE accumulation and diabetes complications from experimental animal studies can be

convincingly confirmed in humans and agents to reduce AGE accumulation become available for daily clinical practice, then decreasing AGE accumulation could become a treatment goal. However at this point AGE accumulation is mainly a means to pinpoint patients with longstanding hyperglycemic burden. And unfortunately the AGE-reader cannot be used to detect milder forms of short-term diabetes such as gestational diabetes.

In conclusion, individualized diabetes management and intervention studies should not only target HbA1c as a parameter, but should also focus on other parameters like glycemic variability, quality of life, insulin dose and potentially AGE accumulation.

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Chapter 11

Summary

Diabetes mellitus is a chronic disease associated with development of microvascular (retinopathy, nephropathy or neuropathy) and macrovascular (cerebrovascular, coronary or peripheral artery disease) complications. Optimal glycemic control is the cornerstone of diabetes management for prevention of diabetes complications and therefore glucose monitoring is needed. To assess glycemic control, usually HbA1c is used, which is a reflection of the mean glucose level in a patient over the past months. Although HbA1c lowering directly results in reduced diabetes complication risk, HbA1c does not provide all information about glycemic control. HbA1c does not provide information about actual glucose levels, glucose peaks and nadirs, whereas glycemic variability does. Furthermore HbA1c provides information about the mean glucose from the last few months, whereas accumulation of advanced glycation end products (AGEs) may serve as a more long-term reflection of hyperglycemia and tissue glycation.

In this thesis glycemic variability and AGEs are investigated as alternative assessment of glycemic control.

Diabetes is not only characterized by hyperglycemia, but also by fluctuating glucose levels (glycemic variability). In normal subjects glucose levels are kept in a very small range, but glycemic variability is much larger in diabetes patients. While HbA1c is a very straightforward measurement, which has been validated all over the world, much more discussion exists about what glycemic variability is and how it should be measured. More than 20 parameters have been described to measure glycemic variability. Studies investigating glycemic variability would obviously benefit from comparability of parameters of this entity. In **chapter 2** we showed that many parameters are highly correlated. We suggest the parameter of choice to be standard deviation of blood glucose (SD), which is easy to measure and to understand and showed strong correlations with the other parameters. Chapter 2 also shows that glycemic variability is higher in type 1 diabetes compared to type 2 diabetes, probably caused by different progression rates of these diseases. In patients with type 2 diabetes glycemic variability did increase, but levels comparable with type 1 diabetes patients were only reached after approximately 20 years of insulin therapy. This emphasizes that large glycemic variability is not only present in type 1 diabetes, but also an important feature of (longstanding) type 2 diabetes on insulin therapy.

If the body puts so much energy in keeping glucose levels in a very stable range in the physiological situation, one could imagine that variability of glucose levels is harmful for diabetes patients. Glycemic variability has indeed

been suggested to be an important risk factor for development of diabetes complications but controversy about this issue remains since data from literature are contradictory. In **chapter 3** we investigated whether a fluctuating glucose level on its own is bothersome for a patient by relating it to quality of life. Our hypothesis was that fluctuating blood glucose levels would result in more problems with treatment and negative feelings due to unpredictable alterations in blood glucose. Glycemic variability is indeed associated with quality of life in patients with type 2 diabetes, measured by the disease-specific Problem Areas in Diabetes Scale (PAID), especially in the domains of the PAID questionnaire concerned with diabetes-related emotional problems and treatment-related problems. The association was only present in type 2 diabetes patients on insulin use and not independent from HbA1c.

Glycemic variability could theoretically be reduced by extensive glucose monitoring, enabling patients to react to minor glucose changes, and in doing so keeping variability as low as possible. The real-time continuous glucose monitoring (which is a system containing a needle which has to be inserted into subcutaneous fat and displays the glucose profile to the patient) is the most useful device for this purpose since it can be used continuously to improve glycemic control. **Chapter 4** describes a systematic review about the use of the real-time continuous glucose monitoring for the treatment of diabetes. This review not only showed that this device has the ability to decrease HbA1c, but also the potential to reduce glycemic variability.

Another possibility to reduce glycemic variability is intervening in the incretin pathway (by DPP4-inhibitors), since these agents have effects on alpha cell as well as on the beta cell function. An increase in insulin response after a meal (beta cell function) together with a decrease in postprandial glucagon (alpha cell function) could reduce hyperglycemic excursions, while an increase in glucagon response following a hypoglycemia could reduce a hypoglycemic excursion. Altogether these effects of DPP4-inhibitors could in theory reduce glycemic variability. This hypothesis was investigated in **chapter 5** in a small randomized study with addition of vildagliptin (a DPP4-inhibitor) to start of insulin treatment in patients with type 2 diabetes. This study eventually failed to reach the calculated sample size due to insolvable recruitment problems. This study does therefore not provide a definite answer if a DPP4 inhibitor reduces required insulin dose or glycemic variability, but should be considered as a small pilot study, which can be used to base a power calculation for a larger intervention trial on.

HbA1c is just a reflection of the mean glucose and does not provide information about actual glucose levels as described above. HbA1c is also only a reflection of the glucose levels over the past months, whereas measurement of accumulation of advanced glycation end products (AGEs) can provide information about hyperglycemia for a much longer period of time. AGEs are modified (i.e. glycated) long-lived tissue proteins that accumulate in body tissue during aging and accelerated accumulation is seen in patients with diabetes. Accumulation of AGEs can be harmful by interaction with the receptor for AGEs or by directly altering proteins, for instance accumulation of AGEs in the extracellular matrix can reduce elasticity and lead to arterial stiffness. A pathophysiological role for AGE-accumulation has been confirmed in experimental studies, although this could not be convincingly or safely corroborated in intervention studies in humans. AGEs are thought to have a role in the concept of "metabolic memory". Metabolic memory means a long-lasting detrimental effect on tissues of longstanding hyperglycemia continuing after improvement of glycemic control. AGEs can be measured in skin biopsies or in blood, which are invasive methods to measure AGEs, whereas AGE-accumulation can easily be assessed non-invasively by the AGE-reader. This device uses auto-fluorescent characteristics of AGEs to measure skin autofluorescence (SAF) as an estimation of the AGE accumulation in the skin of a patient. SAF can be measured by the AGE-reader requiring the patient to position the volar side of the arm on the device.

First of all existing evidence about the AGE-reader and diabetes complications in patients with type 1 or type 2 diabetes was reviewed in **chapter 6**. This review demonstrates that all included studies showed associations of AGE accumulation with diabetes complications. However, not all studies investigated all diabetes complications and different studies showed different results. For instance, no association was found for elevated AGEs and retinopathy, whereas recent literature does describe such an association. To investigate these issues more thoroughly an observational study was performed in our own large cohort of type 2 diabetes patients investigating the association of skin autofluorescence measured by the AGE-reader and different diabetes complications (**chapter 7)**. Accumulation of AGEs was associated not only with neuropathy and nephropathy, but also with retinopathy, albeit dependent on the stage (and therefore definition) of retinopathy (the association was only found for proliferative retinopathy). AGEs were also associated with peripheral artery disease and AGE accumulation increased with increasing number of complications. SAF was not only associated with neuropathy and nephropathy as such, but also linearly with the severity of these complications.

In contrast to the extensively investigated associations of AGE accumulation with diabetes complications, the use of the AGE-reader was never investigated in gestational diabetes mellitus. To detect gestational diabetes an oral glucose tolerance test is used, which is an invasive and laborious test. This oral glucose tolerance test is performed in all women with maternal risk factors for gestational diabetes. If AGE-accumulation is present in gestational diabetes patients, the non-invasive AGE-reader could possibly be used to this purpose instead of an oral glucose tolerance test. As a first step to evaluate if the AGE-reader could be used to detect gestational diabetes we evaluated if accelerated AGE-accumulation, measured as skin autofluorescence was present in gestational diabetes patients in **chapter 8**. No difference in AGE accumulation was found between patients with gestational diabetes mellitus and pregnant women without diabetes. Therefore the AGE-reader cannot be used to detect gestational diabetes. This was unexpected since the hypothesis was that patients, who develop GDM are metabolically different from patients who do not. Apparently the disease is too mild and of too short duration to cause AGE-accumulation at diagnosis.

In contrast, in **chapter 9** we showed that skin autofluorescence was higher in pregnant patients with pre-existent diabetes (type 1 or type 2 diabetes), in whom glucose levels are also elevated before pregnancy. Moreover, it was unexpectedly found in this study that SAF slightly decreased in normal pregnancy in contrast to pregnancy complicated by diabetes. This is remarkable in view of the fact that skin collagen has a half-life of approximately 15 years. Local factors like edema could have played a role or pregnancy-related faster protein turn-over of skin collagen. However, a definitive explanation can only be speculated on.

In conclusion, AGE accumulation has the ability to pinpoint patients with long-term glycemic burden, since it reflects hyperglycemia for a much longer period than Hba1c. AGE accumulation can play a pathophysiological role in diabetes complications. However, human intervention studies have so far not convincingly confirmed this pathophysiological role. We showed that AGE accumulation was associated with all different diabetes complications and hence could be part of a final common pathway in causing these complications. The AGE-reader cannot be used to detect patients with milder forms of diabetes such as gestational diabetes. Apparently these patients have no hyperglycemic burden yet, at the moment that gestational diabetes is detected.

It can be debated if HbA1c should be the only main treatment target in daily clinical practice. Not all patients will benefit from HbA1c lowering, since it is an oversimplification of the actual glycemic control. Glycemic variability should be used as an additional treatment goal, since lowering of Hba1c will otherwise inevitably result in more hypoglycemia. We showed that glycemic variability is directly associated to quality of life, hence glycemic variability should be considered an important patient-related end-point. Fortunately, many of the recent developments in measurement of glucose (real time continuous glucose monitoring), as well as additions to the therapeutic arsenal (DPP4-inhibitors) have the ability to decrease glycemic variability in diabetes patients.

Chapter 11

Nederlandse samenvatting

Diabetes mellitus is een chronische ziekte waarbij patiënten diabetische complicaties kunnen ontwikkelen. Deze bestaan uit microvasculaire complicaties; retinopathie (aandoening van de ogen), neuropathie (aandoening van de zenuwen), nefropathie (aandoening van de nieren) en uit macrovasculaire complicaties (hartaanval, beroerte of perifeer vaatlijden). Optimale glycemische controle is de basis van diabetes behandeling om complicaties te voorkomen. Daarom is het nodig dat glucose waardes gecontroleerd worden. HbA1c (een weergave van de gemiddelde glucose waardes van de afgelopen maanden) wordt normaliter gebruikt om glycemische controle te beoordelen. Hoewel verlaging van het HbA1c direct leidt tot minder diabetes complicaties, geeft HbA1c niet alle informatie over glycemische controle weer. HbA1c geeft namelijk geen informatie over de werkelijke glucose waardes, glucose pieken en dalen. Terwijl glycemische variabiliteit hier wel informatie over verschaft. Verder is HbA1c een weergave van de gemiddelde glucose waardes van de afgelopen maanden, terwijl advanced glycation end products (AGEs) een weergave zijn van de lange termijn hyperglycemie en glycosilering van de weefsels.

In dit proefschrift worden glycemische variabiliteit en AGEs onderzocht als alternatieven voor evaluatie van glycemische controle.

Diabetes wordt niet alleen gekenmerkt door hyperglycemie, maar ook door wisselende glucose waardes (glycemische variabiliteit). Bij personen zonder diabetes houdt het lichaam de glucose waardes in het bloed binnen erg smalle grenzen. De glycemische variabiliteit is vele malen groter bij diabetes patiënten. HbA1c is een meting, die internationaal overal gelijk en gevalideerd is. Er bestaat echter nog veel discussie over wat glycemische variabiliteit nu precies inhoudt en hoe deze gemeten zou moeten worden. Er zijn meer dan 20 parameters beschreven om glycemische variabiliteit weer te geven. Studies over variabiliteit zouden gebaat zijn bij één vergelijkbare parameter om variabiliteit te beschrijven. In **hoofdstuk 2** werden naast standaard deviatie (SD) van de bloedglucose waardes een aantal andere parameters voor variabiliteit gemeten. De standaard deviatie van bloedglucosewaardes is een makkelijk te meten en begrijpelijke weergave van glycemische variabiliteit. Deze was in onze studie sterk gecorreleerd aan de andere gemeten parameters en heeft daarom de voorkeur om als standaard parameter voor glycemische variabiliteit te worden gebruikt. Verder wordt in hoofdstuk 2 beschreven, dat glycemische variabiliteit hoger is in patiënten met type 1 diabetes dan in patiënten met type 2 diabetes. Dit heeft te maken met de verschillen in ziekteprogressie. In patiënten met type 2 diabetes nam de glycemische variabiliteit wel geleidelijk toe, maar deze was pas na ongeveer 20 jaar insuline therapie gelijk aan de variabiliteit van patiënten met type 1 diabetes. Dit benadrukt, dat glycemische variabiliteit niet alleen van

belang is voor patiënten met type 1 diabetes, maar ook bij patiënten met lang bestaande type 2 diabetes, die behandeld worden met insuline.

Als het lichaam er zoveel energie in steekt om bloedglucosewaardes binnen zeer smalle grenzen te houden in de normale situatie, dan lijkt het logisch dat variatie in glucosewaardes schadelijk is voor diabetes patiënten. Glycemische variabiliteit wordt inderdaad in verband gebracht met het ontstaan of verergeren van diabetes complicaties. Toch blijft er controverse bestaan over dit onderwerp, omdat studies tegenstrijdige resultaten laten zien. In **hoofdstuk 3** werd onderzocht of een fluctuerende glucose waarde op zich hinderlijk is voor een patiënt met diabetes door glycemische variabiliteit te relateren aan kwaliteit van leven. De hypothese hierbij was dat sterk fluctuerende glucoses zorgen voor negatieve emoties bij diabetes patiënten over deze onvoorspelbare wisselingen en daardoor voor meer problemen bij de behandeling. In hoofdstuk 3 laten we inderdaad een verband zien tussen glycemische variabiliteit bij patiënten met type 2 diabetes en hun kwaliteit van leven, gemeten met de "probleemgebieden bij diabetes" vragenlijst (PAID). Patiënten met een hogere glycemische variabiliteit hadden een slechtere score in de gebieden van de PAID vragenlijst die te maken hadden met diabetes gerelateerde emotionele problemen en met aan de behandeling gerelateerde problemen. Het verband was alleen aanwezig bij patiënten met type 2 diabetes, die ook insuline gebruikten en niet onafhankelijk van HbA1c.

Glycemische variabiliteit zou theoretisch verlaagd kunnen worden door real-time continue glucose monitoring (een systeem dat via een klein naaldje in de buikhuid continue de glucose meet en dit op een schermpje laat zien aan de patiënt). Dit zorgt ervoor dat patiënten al snel kunnen reageren, ook op kleine wisselingen in bloedglucosewaardes waardoor ze de glycemische variabiliteit kunnen verlagen. Het review over het gebruik van real-time glucose monitoring bij de behandeling van diabetes in **hoofdstuk 4** laat niet alleen zien dat het HbA1c hiermee verlaagd kan worden, maar toont bovendien een potentieel effect van real-time continue glucose monitoring op glycemische variabiliteit.

Een andere mogelijkheid om glycemische variabiliteit te verlagen zou interventie in het incretine systeem (met DPP4-remmers) kunnen zijn, omdat deze middelen effecten hebben op zowel alpha- als bètacelfunctie. Een toename van de insulineafgifte na een maaltijd (bètacel functie), samen met minder afgifte van glucagon na een maaltijd (alphacel functie) kan leiden tot minder hyperglycemieën. Terwijl een betere glucagon afgifte bij een hypoglycemie (alphacel functie) de hypoglycemie juist weer kan beperken. Uiteindelijk zouden deze effecten van DPP4-remmers tezamen kunnen zorgen voor minder glycemische variabiliteit. Deze hypothese werd onderzocht in

hoofdstuk 5, in een kleine gerando-miseerde studie naar toevoeging van vildagliptine (een DPP4-remmer) aan het begin van de insuline behandeling bij patiënten met type 2 diabetes. Helaas bleek het erg moeilijk om patiënten te vinden die voor deze trial in aanmerking kwamen. Daardoor kon de berekende groepsgrootte uiteindelijk niet worden gehaald. Hierdoor is het niet mogelijk om een definitief antwoord te geven op de vraag of toevoeging van een DPP4-remmer in deze groep de benodigde hoeveelheid van insuline (aantal eenheden nodig voor glycemische controle) of de variabiliteit vermindert. Deze studie kan wel gebruikt worden als een kleine pilot studie, waarop een grotere trial zich dan weer kan baseren.

Zoals hierboven beschreven is HbA1c slechts een weergave van alleen de gemiddelde bloedglucosewaardes en niet van de actuele wisselingen in glucosewaardes. Daarnaast geeft HbA1c ook slechts een gemiddelde waarde van de afgelopen maanden. Terwijl meting van de accumulatie van advanced glycation end products (AGEs) wel wat zegt over de hyperglycemie over een langere periode. AGEs zijn gemodificeerde eiwitten die accumuleren in verschillende weefsels bij veroudering en die versneld ontstaan bij patiënten met diabetes. AGEs kunnen schade veroorzaken via interactie met de receptor voor AGEs of door directe verandering van eiwitten (accumulatie van AGEs in de extracellulaire matrix kan bijvoorbeeld leiden tot minder elasticiteit van de bloedvaten). Een pathofysiologische rol bij het ontstaan van complicaties is aangetoond in dierproeven, hoewel dit niet overtuigend kon worden bevestigd in humane studies. AGEs spelen mogelijk een rol bij het metabole geheugen (het feit dat slechte glycemische controle een zeer langdurig negatief effect heeft op het ontstaan van complicaties, ook nadat deze controle is verbeterd). AGEs kunnen worden gemeten in huid biopsieën en in bloed, maar het is ook mogelijk AGEs non-invasief te meten. Dit kan met de AGE-reader, een apparaat dat gebruik maakt van de autofluorescerende eigenschappen van AGEs en dit omzet naar een huid-autofluorescentie van een patiënt (skin autofluorescnece (SAF)). De SAF is een maat voor de accumulatie van AGEs in de huid en kan gemeten worden door de patiënt de onderarm op de AGE-reader te laten plaatsen.

Hoofdstuk 6 beschrijft een literatuur studie over de AGE-reader en complicaties bij patiënten met diabetes. Dit systematische review leverde 7 artikelen op, die allemaal een relatie tussen verhoogde AGEs en diabetes complicaties lieten zien. Echter de meeste studies bekeken niet alle verschillende complicaties. Ook lieten verschillende studies, verschillende resultaten zien. Er werd bijvoorbeeld geen associatie tussen AGEs en retinopathie gevonden in de studies in ons

review. Recentere literatuur beschrijft dit wel. Om dit verder te onderzoeken werd in **hoofdstuk 7** de relatie tussen AGEs en diabetes complicaties in ons eigen cohort van patiënten met type 2 diabetes onderzocht (Parelsnoer onderzoek)**.** Niet alleen bleek accumulatie van AGEs gerelateerd aan neuropathie en nefropathie, maar ook aan retinopathie. Al was dat erg afhankelijk van de ernst en de manier waarop retinopathie werd gedefinieerd (de relatie werd alleen gevonden voor proliferatieve (ernstige) retinopathie). Tevens werd in onze studie een relatie tussen AGEs en perifeer arterieel vaatlijden gevonden. Voorts was een hogere SAF-waarde (meer AGE accumulatie) direct geassocieerd met meer microvasculaire complicaties. SAF was niet alleen gerelateerd aan neuropathie en nefropathie op zich, maar er was tevens een lineair verband tussen AGE accumulatie en de ernst van de neuro- of nefropathie.

In tegenstelling tot de relatie tussen AGE accumulatie en diabetes complicaties, waar veel onderzoek naar is gedaan, was het nut van de AGE-reader bij zwangerschapsdiabetes nog nooit onderzocht. De diagnose zwangerschapsdiabetes wordt normaliter met een orale glucose tolerantie test gesteld. Dit is een invasieve test, waarbij er bij patiënten bloed wordt afgenomen, na inname van een suikerhoudende drank. Op dit moment wordt bij alle vrouwen met risicofactoren voor zwangerschapsdiabetes een dergelijke orale glucose tolerantie test uitgevoerd. Echter, als AGE accumulatie aanwezig is bij patiënten met zwangerschapsdiabetes dan zou de non-invasieve AGE-reader meting misschien gebruikt kunnen worden in plaats van de orale glucose tolerantie test. Er was nog niets bekend over AGE accumulatie in de zwangerschap, daarom hebben we eerst onderzocht of huid-autofluorescentie (AGE accumulatie) van patiënten met zwangerschapsdiabetes hoger is dan bij zwangeren zonder diabetes (**hoofdstuk 8)**. Er werd echter helemaal geen verschil gevonden in huid-autofluorescntie tussen zwangeren met en zonder zwangerschapsdiabetes. Daarom kan de AGE-reader niet worden gebruikt om patiënten met zwangerschapsdiabetes op te sporen. Dit was een onverwachte bevinding, aangezien we hadden verwacht dat patiënten die zwangerschapsdiabetes krijgen toch metabool anders zijn dan zwangeren die dit niet krijgen. Blijkbaar is de hyperglycemie bij patiënten met zwangerschapsdiabetes toch te mild en te kort om AGE accumulatie te veroorzaken.

In **hoofdstuk 9** wordt beschreven dat de AGE accumulatie wel duidelijk verhoogd is bij zwangere patiënten met type 1 of type 2 diabetes, dus patiënten die al diabetes hadden, voordat ze zwanger waren. Bovendien was een onverwachtse uitkomst, dat de huid-autofluorescentie licht daalde tijdens de zwangerschap

bij gezonde zwangere vrouwen. Terwijl dit niet het geval was bij zwangeren met diabetes. Omdat de halfwaardetijd van huidcollageen (het weefsel waar AGEs accumuleren in de huid) veel langer is (ongeveer 15 jaar) kunnen we deze lichte daling niet verklaren. Maar mogelijk spelen lokale factoren, zoals oedeem of een snellere turnover van eiwit tijdens de zwangerschap een rol.

Concluderend, patiënten met lange termijn (hyperglycemische) schade kunnen worden gedetecteerd met behulp van AGE accumulatie, omdat dit een weergave is van hyperglycemie over een veel langere termijn dan HbA1c. Bovendien kan AGE accumulatie een rol spelen in het ontstaan van complicaties. Echter interventie studies in mensen hebben deze pathofysiologische rol tot nu toe niet overtuigend kunnen bevestigen. AGE accumulatie is geassocieerd met alle verschillende diabetes complicaties en zou daarom deel kunnen zijn van een "final common pathway". De AGE-reader kan niet gebruikt worden om patiënten met een mildere vorm van diabetes (zwangerschapsdiabetes) op te sporen. Blijkbaar is de blootstelling aan hyperglycemie bij deze patiënten op het moment van diagnose nog onvoldoende om dit te detecteren.

Het is de vraag of HbA1c het belangrijkste behandeldoel is en moet blijven in de dagelijkse klinische praktijk. Niet alle patiënten zullen baat hebben bij HbA1c verlaging, omdat HbA1c een simplificatie van de werkelijkheid is. HbA1c verlaging zal resulteren in meer hypoglycemieën als niet tegelijkertijd een reductie van de glycemische variabiliteit wordt nagestreefd. Glycemische variabiliteit is direct gerelateerd aan kwaliteit van leven en daarmee is dit tevens een belangrijk patiënt-gerelateerd eindpunt. Gelukkig zijn een aantal van de recente ontwikkelingen op gebeid van glucose monitoring ("real time" continue glucose monitoring) en toevoegingen aan het therapeutisch arsenaal (zoals DPP4-remmers) in staat deze glycemische variabiliteit te verlagen.

Chapter 12

Dankwoord

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Wendela de Ranitz-Greven

Chapter 12

Curriculum vitae

Wendela Lucia Greven was born 29th of July 1980 in Amsterdam. She grew up in Enschede and finished her secondary school in 1998. That year she started to study Pharmacy at the University of Groningen. After her propaedeutics in Pharmacy she started to study Medicine (1999) and graduated in 2005.

From 2002 – 2005 she participated in a research project "Advanced Glycation End products in renal disease" at the department of Nephrology and Pathology under supervision of Prof. dr. G.J. Navis and Prof. dr. H. van Goor. Part of her research project was performed in the laboratory of Prof. dr. J.W. Baynes and Dr. S.R. Thorpe (Columbia, South Carolina, USA), where she worked 3 months on biochemical detection of AGEs .

In 2005 she moved from Groningen to Utrecht and started her residency Internal Medicine at the University Medical Centre of Utrecht (UMCU). The first two years of this residency she worked in Gelre ziekenhuizen Apeldoorn (Dr. J.M. Smit, Dr. C.G. Schaar). From December 2007 on she continued her residency in the UMCU (Prof. dr. E. van der Wall, Prof. dr. D.H. Biesma, Prof. dr. M.M.E. Schneider). In 2012 she started her specialization Endocrinology (Dr. P.M.J. Zelissen, Prof. dr. G.D. Valk).

During her residency she started with clinical research for this thesis "Alternative Assessment of Glycemic Control" under supervision of Prof. dr. D.H. Biesma, Dr. H.W. de Valk and Dr. J.W.J. Beulens and worked from 2009-2011 on this project full-time. From 2011 she combined her residency with research work for her thesis.

In 2010 Wendela married Stan de Ranitz and they have two children; Florens (2011) and Bastiaan (2013).

Chapter 12

List of publications

Is higher glycemic variability associated with reduced quality of life in type 2 diabetes patients? **de Ranitz-Greven WL**, Beulens JWJ, Biesma DH, Silvius B, Bons M, de Valk HW. *Submitted.*

Accumulation of advanced glycation end products, measured by skin autofluorescence is associated with presence and number of diabetes complications; a cross-sectional study. **de Ranitz-Greven WL**, Beulens JWJ, Biesma DH, Silvius B, Bons M, de Valk HW. *Submitted.*

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