Chapter 9

General Discussion

Judith C. Kuenen

The ADAG study (Chapter 2)

The A_{1c} Derived Average Glucose (ADAG) study [1] was an international multicenter study (2006-2008) which examined the relationship between the average blood glucose concentrations (over 3 months) and HbA_{1c} in a diverse population and to determine whether HbA_{1c} could be expressed and reported as average glucose in the same units as used in self-monitoring. Further we examined the influence of factors such as age, gender, race (Caucasian, African or African American, or Hispanic), and smoking history on the relationship between HbA_{1c} and mean blood glucose (MBG). A total of 507 participants from 10 international centers completed 3 months of frequent glucose monitoring.

Major findings

The ADAG study showed a tight linear relationship between HbA_{1c} and MBG in both T1DM and T2DM patients ($R^2 = 0.84$). This relationship was earlier described in the Diabetes Control and Complications Trial (DCCT) study (R = 0.82) [2], and by Nathan (R = 0.90) but only in T1DM patients [3]. Furthermore the DCCT included 1441 patients with T1DM and followed them several years to monitor complications, however these patients performed 7-point blood glucose profiles only once during 3-months. Nathan included only 22 patients with T1DM and 3 patients without Diabetes Mellitus (DM), who performed continuous glucose monitoring (CGM) during a 3-month period.

The ADAG study was designed to establish the relationship between HbA_{1c} and MBG and had the important advantages of frequent blood glucose monitoring allowing for an accurate determination of MBG in a more diverse population as we included different ethnic groups. A total of 507 participants (268 T1DM, 159 T2DM, and 80 non-DM) were included and approximately 2700 glucose values per patient that captured a median of 52 days, were measured in a 3-month study period. Furthermore four DCCT aligned HbA_{1c} measurement methods were used and all samples were measured in a central laboratory. These are the likely explanations for the less wide scatter around the regression line, suggestive of a higher precision, and for the lower estimated Average Glucose (eAG) values,

compared with the widely used equation derived from the earlier mentioned DCCT study.

Age, gender, racial and ethnic differences

The linear regression equations did not differ significantly across subgroups based on age, gender, diabetes type, race/ethnicity, or smoking status.

Age and gender: A meta-analysis of data from the Framingham Offspring Study and the National Health and Nutrition Examination Survey showed that in non-diabetic persons HbA_{1c} values gradually increase by approximately 7 mmol/mol HbA_{1c} (0.6%) between the ages of 40 and 70 years, reflecting the change in average glycemia with age [4]. Other studies confirm the positive association between age and HbA_{1c} in adults [5, 6]. Faerch et al. and Gulliford et al. both found somewhat higher levels of HbA_{1c} in men compared to women [7, 8], but other studies found no gender-related differences in HbA_{1c} [9, 10].

Race/ethnicity: The results of the ADAG trial suggested (P = 0.07) that the regression line was different for African Americans such that for a given value of HbA_{1c}, African Americans might have a slightly lower mean glucose level. The African and Indian ethnic groups were unfortunately underrepresented in the ADAG study. The latter was mainly due to one of the South-East-Asian centers withdrawing from the study due to technical difficulties. The influence of ethnicity on the MBG-HbA_{1c} relationship therefore requires further studies.

Recently, racial and ethnic differences in the relationship between HbA_{1c} and blood glucose have been reported [11-13]. Ziemer et al. found higher HbA_{1c} levels in black persons than in white persons across the full spectrum of glycemia after adjustments for plasma glucose and other characteristics known to correlate with HbA_{1c} levels [14]. And also, subjects of South Asian origin showed to have higher HbA_{1c} levels than white subjects independent of fasting and post-load glycemia during an oral glucose tolerance test (OGTT) [15].

The results of the Diabetes Prevention Program (3819 individuals ≥ 25 years old with impaired glucose tolerance (IGT)) indicate that ethnicity is an independent factor in determining HbA_{1c}: 'Adjusting for glucose concentration and a range of other factors, mean HbA_{1c} levels were 5.78% for whites, 5.93% for Hispanics, 6.00% for Asians, 6.12% for American Indians, and 6.18% for African Americans ($p < 0.001$) [13]. Although the potential reasons for racial and ethnic differences remain unknown, factors such as differences in red cell survival, extracellular-intracellular glucose balance, and non-glycemic genetic determinants of hemoglobin glycation are being explored as possible contributors. Also the way MBG was assessed, e.g. pre- versus post meal blood glucose measurements will affect the MBG estimation and thereby the assessment of the relationship with HbA_{1c}.

Until the reasons for these differences are more clearly defined, reliance on HbA_{1c} as the sole, or even preferred, criterion for the diagnosis of diabetes creates the potential for systematic error and misclassification. HbA_{1c} must be used thoughtfully and in combination with traditional glucose criteria when screening for and diagnosing diabetes.

There is growing literature describing measures of glycemic control in specific racial/ethnic groups, and the differences among groups [13]. One study found racial/ethnic differences in HbA_{1c} and 1,5 AnhydroGlucitol (1,5AG) that could not be attributed to MBG [16]. These data raise questions about concordance of self-monitoring of blood glucose (SMBG), HbA_{1c}, and 1,5AG, and highlight the need to better understand factors that could influence each of these parameters before comparisons of these measures across different racial/ethnic groups can be considered reliable. Of course these results cannot be compared to the ADAG study as these studies were not primarily designed to assess the MBG-HbA_{1c} relationship and were not able to obtain a reliable measure of MBG.

Currently, HbA_{1c} is the primary marker of glycemic control in patients with DM, primarily because of the strong predictive relationship with long-term complications. However, the ADAG study and other more recent findings suggest the potential for using multiple measures of

glycemia, e.g. 1,5AG and glucose variability (GV) to improve our understanding of overall glycemic control across diverse populations [16].

Smoking and alcohol consumption: A negative association between alcohol consumption and HbA_{1c} has been found in at least three studies regarding the association between alcohol consumption and HbA_{1c} [7, 17, 18]. In contrast, Meyer et al. could not confirm these findings in their study to the relations of alcohol patterns with HbA_{1c} in non-diabetic men [19]. Several studies have documented that smoking is associated with higher HbA_{1c} levels [7, 10, 20, 21], but Koga et al. found no association between smoking and HbA_{1c} levels [22]. Glycotoxins found in cigarette smoke may induce the higher rate of glycation of HbA [23] or the relative higher tissue hypoxia [24] can explain increased HbA_{1c} levels in smokers [25].

Limitations

The ADAG study has a few limitations. In contrast to our intention and expectation, some ethnic/racial groups were underrepresented, primarily because of the withdrawal of one of the centers with a large Asian population and a limited number of subjects of African descent.

In addition, the average glucose estimation was based predominantly on two methods: continuous glucose monitoring (CGM) and intermittent SMBG. (The Hemocue measurements, recognized as providing values that are equivalent to laboratory measurements, were used primarily to calibrate the CGM.) To combine these measurements into a single calculated eAG, the CGM and finger-stick capillary measurements had to be weighted to take into account the different number of measurements in a day; however, in separate analyses comparing the relationships between HbA_{1c} and eAG measured with CGM or finger stick capillary measurements, there was no significant difference in the relationships.

Finally, since only diabetic patients in stable glycemic control and without any suggestion of erythrocyte disorders were entered into the study, the current results are only directly applicable to this population.

Persons with clinical conditions that potentially could affect HbA_{1c} results by affecting erythrocyte lifespan, were excluded from the ADAG study. These included pregnant women, persons with hematological conditions (e.g. anemia, hemoglobinopathies, blood loss) and those with severe renal or liver disease. It has been argued that additional data in these groups are needed to confirm the established MBG-HbA_{1c} relationship. However, for this to be carried out a more complex design and logistics of the measurements would be required. Accordingly, glucose monitoring periods would have to be planned at specific stages of pregnancy, and at specific levels of anemia, renal disease etc. Such an approach is challenging and may not be feasible. Instead emphasis should be placed on the fact that the glycation process depends on erythrocyte lifespan – no matter what assay or units are implemented.

Conclusions

We concluded that HbA_{1c} levels could be expressed as eAG for most patients with T1DM and T2DM and patients without DM. The MBG-HbA_{1c} relationship in non-Caucasian groups and in young patients should be examined further.

Implementation of IFCC HbA_{1c} test results

Amongst the important diabetes organizations and the American Association for Clinical Chemistry (AACC) there is consensus that HbA_{1c} should be reported in both National Glycohemoglobin Standardization Program (NGSP) units in % and International Federation of Clinical Chemistry (IFCC) units in mmol/mol along with eAG in either mmol/L or mg/dL)[26] (www.aacc.org/gov/gov_affairs/positions/pos_stat_09/Documents/AACC Position eAG.pdf). Table 1 depicts the National Glycohemoglobin Standardization Program (NGSP) standardized HbA_{1c} values and the eAG in mmol/L and mg/dL for different given IFCC-HbA_{1c} values.

However, the final decision on what to report and to whom is being made country by country. Some countries decided not to report 3 different test results per patients. Other associations were not convinced about the clinical benefit of reporting eAG in clinical practice, mainly because of the wide eAG range for a given HbA_{1c} level. Indeed, the regression line from

the ADAG study demonstrated a wide range of average glucose levels for individuals with the same HbA_{1c} level (Fig. 1). An HbA_{1c} value of 6.0% corresponds to an eAG of 5.5 – 8.5 mmol/l (100–152 mg/dl), and an HbA_{1c} value of 7.0% corresponds to an eAG of 6.8 – 10.3 mmol/l (123–185 mg/dl) (95% confidence intervals) [1].

Table 1: NGSP standardized HbA_{1c} values and the eAG in mmol/L and mg/dL for different IFCC-HbA_{1c} values

IFCC-HbA_{1c} (mmol/mol)	**NGSP-HbA_{1c} (%)**	**eAG (mg/dL)**	**eAG (mmol/l)**
31	5	97	5.4
42	6	126	7.0
53	7	154	8.6
64	8	183	10.2
75	9	212	11.8
86	10	240	13.4
97	11	269	14.9
108	12	298	16.5

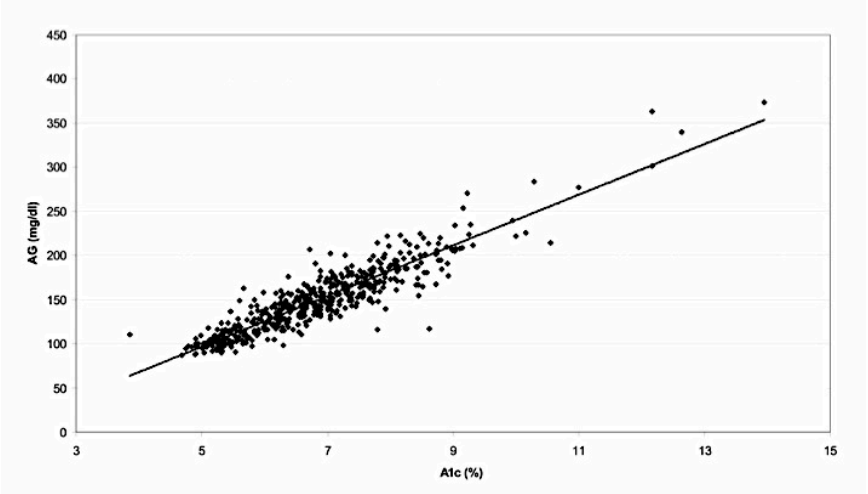

Figure 1: Linear relationship between estimated Average glucose (eAG over 3 months) and HbA_{1c} at the end of the 3 months [1].

In the US, reporting NGSP % HbA_{1c} along with eAG has been recommended by the American Diabetes Association (ADA) and the AACC. Most countries report IFCC and NGSP and some switched to IFCC only. Notwithstanding the different numbers, reported results will always be traceable to the anchor IFCC assay. (Table 2) The ADA, International Diabetes Federation (IDF), European Association for the Study of Diabetes (EASD), and International Society Pediatric and Adolescent Diabetes (ISPAD) as well as other member associations in different countries currently provide patient care guidelines that relate directly to the DCCT aligned NGSP numbers. These will need to be updated to include both NGSP and IFCC reference values.

As stated earlier, the aim was to report the HbA_{1c} as eAG, in the same units as used for day-to-day monitoring to facilitate the interpretation in routine clinical care, but this failed. Unfortunately, the worldwide standardization and the implementation on how to report HbA_{1c} test results has not been successful. The reporting and interpretation of HbA_{1c} of clinical data and research results in the diabetes field worldwide has become more complicated, but the comparability of assay's worldwide has improved as they are all traceable to the anchor IFCC assay.

IFCC Network as an NGSP anchor

The IFCC network is now a secondary anchor for the NGSP. The stability of the relationship over time between the IFCC and NGSP will continue to be monitored. The NGSP certification process will not change and an IFCC Laboratory Network has been established. The lists of current approved and candidate IFCC Network Laboratories can be found at: http://www.ifcchba1c.net/. Table 2 shows the conversion factors for IFCC compared to each of the designated comparison methods (DCMs) including the NGSP.

Table 2: Conversion factors for IFCC compared to each of the designated comparison methods (DCMs) including the NGSP.

DCM **From IFCC to DCM**	**From DCM to IFCC**
NGSP (USA) NGSP = (0.09148*IFCC) + 2.152	IFCC = (10.93*NGSP) - 23.50
JDS/JSCC (Japan) JDS = (0.09274*IFCC) +1 .724	IFCC = (10.78*JDS) - 18.59
Mono-S (Sweden) Mono-S = (0.09890*IFCC) + 0.884	IFCC = (10.11*Mono-S) - 8.94

Glucose Variability and HbA_{1c} (Chapter 3)

Glucose Variability

The potential contribution of GV or postprandial hyperglycemia to the Hemoglobin glycation process is still unclear. Fasting and postprandial glucose (PPG) excursions both contribute to the MBG or total glucose exposure, and therefore to HbA_{1c} [27]. The specific question is whether PPG and GV, apart from the contribution to MBG, also affect the glycation process and therefore may affect the relationship between MBG and HbA_{1c}.

Assessing Glucose Variability

There are different methods to quantify GV. In the ADAG study we calculated several variability metrics, as for example "Amplitudes of glycemic excursions", the Standard Deviation (SD) of all blood glucose values, and the Magnitude of the Amplitude of Glycemic Excursions (MAGE) as described by Service et all [28]. Furthermore we applied the Continuous Overlapping Net Glycemic Action (CONGA) to calculate the GV from CGMS. The CONGA is defined as the standard deviation of the differences, and measures the overall intra-day variation of glucose recordings [29].

Influence of glucose variability on the MBG-HbA$_{1c}$ relationship

In **Chapter 3** we examined the influence of GV on the MBG-HbA$_{1c}$ relationship. We found that all GV measures significantly, although modestly, influenced the MBG-HbA$_{1c}$ relationship. The variability measure SD showed the strongest influence. High GV (SD) was associated with higher HbA$_{1c}$ levels for a given MBG, and this effect was more pronounced at higher HbA$_{1c}$ levels. However the magnitude of this effect of GV was small and only demonstrable in patients with T1DM. Possibly, the T2DM patient group was too small and the variability in this group too low to find this interaction.

Our results are in line with the results of the DirectNet study in children [30]. Although the authors also concluded that HbA$_{1c}$ directly reflects mean glucose over time, they found substantially greater inter-individual variation in the relationship between MGB and HbA$_{1c}$ than in our and Nathan's study in adult patients with T1DM [3]. The DirecNet study used a non-centralized HbA$_{1c}$ method with relative poor correlation with a high-performance liquid chromatography (HPLC) method, and only performed CGM for 67% of the study period, compared to 97% of the 12-week study performed by Nathan [3]. In addition, these children had highly variable glycemia, which will have affected the accuracy of capturing mean glycemia. With high GV it may be difficult to capture the real MBG that determines the measured HbA$_{1c}$ value, as the timing of the assessment in relation to the HbA$_{1c}$ measurement will be critical.

Given the slow kinetics of glycation, brief periods of hyperglycemia should theoretically not have a major impact on HbA$_{1c}$ levels. Previous studies have examined whether the relationship between MBG levels and HbA$_{1c}$ is influenced by GV and found no or minimal influence [31-33]. However, these studies used limited SMBG data in relatively small numbers of patients to assess mean glucose levels and variability. These limitations affect the precision and accuracy of the estimations of MBG and of the glycemic excursions. CGM provides the opportunity to assess more precisely glycemic excursions, including the duration and frequency of the excursions, and allows the calculation of different measures of GV.

In general, GV is higher in patients with poor glycemic control and in patients with T1DM than in patients with T2DM, which probably can be attributed to insulin therapy and higher insulin sensitivity. High GV may affect glycation by exposing the red blood cell to periodic high glucose levels, which in turn stimulates oxidative stress and acceleration of irreversible glycation [34-38]. Recently it has been speculated that oxygen free radicals may promote the formation of early glycated proteins [34, 35]. As proposed by Brownlee, hyperglycemia can be the underlying mechanism for an enhanced oxidative stress [36, 37].

High GV and especially postprandial glucose excursions were also previously associated with oxidative stress in T2DM [38]. The activation of oxidative stress, estimated from urinary excretion rates of Isoprostanes, was highly correlated with MAGE calculated from CGM [38]. However, Wentholt et al could not replicate these results in T1DM [39].

Recent evidence suggests that also hypoglycemia may play an important role in the vascular complications of diabetes [40]. Hypoglycemia also causes oxidative stress [41], inflammation [42], and endothelial dysfunction [43]. Oxidative stress is considered the key player in the pathogenesis of diabetes complications [44, 45]. During hyperglycemia, oxidative stress is produced at the mitochondrial level [44], similarly as in hypoglycemia [41]. Therefore, oxidative stress might be considered the common factor linking hyperglycemia, hypoglycemia, and the vascular complications of diabetes. Consistent with this hypothesis is the evidence that hyperglycemia [46] and hypoglycemia both produce endothelial dysfunction and inflammation through the generation of oxidative stress [43]. Endothelial dysfunction and inflammation are well-recognized pathogenic factors for vascular disease, particularly in diabetes [47].

However, Ceriello et al showed that the way in which recovery from hypoglycemia takes place might also have an effect on cardiovascular risk. When recovery from hypoglycemia results in normoglycemia, the deleterious effects of the previous hypoglycemia are mainly counterbalanced, whereas when recovery is obtained resulting in hyperglycemia, endothelial function, oxidative stress, and inflammation are further worsened [48].

The strength of the ADAG study is the great precision with which the MBG was measured in a large number of individuals with and without diabetes using repeated measures. The intensive glucose monitoring using several methods also allowed to explore several approaches to define PPG, and provided sufficient measurements to reliably assess different features of GV.

Limitations

Limitations of the MiniMed CGM system include the inability to measure glucose values below 2.2 mmol/l or above 22.2 mmol/l. As the participants were selected to have stable HbA_{1c} at baseline (defined as a < 1% HbA_{1c} change during the 6 months prior to the study), and remained relatively stable during the study, we may have limited the range of GV compared to the general diabetic population. Despite a stable HbA_{1c} the GV was still considerable among the participants of the ADAG study.

Conclusions

At higher levels of GV the relationship between HbA_{1c} and MBG in patients with T1DM is altered, leading to a higher HbA_{1c} level for a given MBG. However, the impact (near the HbA_{1c} treatment target of 7 %) is only modest. The potential influence of GV on the glycation process, and on HbA_{1c} in particular, is modest. The mechanism needs to be further elucidated.

Are blood glucose concentrations the sole determinant of HbA_{1c} value? (Chapter 4)

Mean blood glucose and HbA_{1c} are tightly related, but inter-individual variability, quantified by the Hemoglobin Glycation Index (HGI), exists and may be attributable to non-glycemic factors affecting glycation. We explored whether non-glycemic factors were associated with HGI.

Seventy-four 74 (14.6%) of the 507 subjects had HbA_{1c} levels outside the 80% prediction band of the relationship between HbA_{1c} and MBG, 44

subjects were high outliers with higher than predicted HbA_{1c} levels, and 30 subjects were low outliers with lower than predicted HbA_{1c} values.

Measures of GV were the main determinants of a high HGI. The GV measures SD, MAGE, and $CONGA_{4,}$ and AUC_{24} and fructosamine explained the largest fraction of the variance of the outlier status for the High outliers, but not Low outlier group. The small number of patients in the latter group may explain this finding.

Smoking status was the next variable explaining outlier status. As described earlier smoking is associated with higher HbA_{1c} levels [7, 10, 20, 21], but other found no association between smoking and HbA_{1c} levels [22]. Smoking history may change RBC turnover.

Finally, diabetes type, Apo-B levels and insulin and lipid treatment were associated with High outliers. However these factors combined explained only 25% of the variance in the MBG-HbA_{1c} relationship for the High outliers. The "non-glucose variables" diabetes type and insulin were not independently associated with HGI. Smoking status, LDL, Apo-B and Apo-B/A1, independent of GV, were related with high HGI.

Fructosamine concentrations measured at baseline (n = 507) were significantly correlated with HGI and outlier status. This suggests that patients with a high HGI and thus a higher than predicted HbA_{1c} also have higher fructosamine levels. This finding supports prior suggestions that the period of glycemic exposure in the few weeks before an HbA_{1c} measurement- as reflected by fructosamine- may play a disproportionate role in the HbA_{1c} value. Alternatively, GV may affect the rate of glycation in general, measured as HbA_{1c} or fructosamine.

As expected, the T1DM group had higher MBG, HbA_{1c} and GV values than T2DM and non-DM groups. This explains the greater variance in the relationships with High HGI. Kilpatrick et al. found that HbA_{1c} values vary markedly between subjects without diabetes, while values within the same individual are very consistent [49]. A potential, unproved explanation for this biological variability is the concept of fast and slow glycation, as described by Hempe [50] and by earlier smaller studies in

people without [49, 51, 52] and with diabetes [53, 54]. Most of these studies suffered from insufficient number of glucose measurements, therefore, discrepancies between HbA_{1c} and MBG could be secondary to an inaccurate appreciation of MBG. The ADAG study included frequent measurements of blood glucose over time, with frequent measurements 52 of 84 days prior to HbA_{1c} measurement. The HbA_{1c} level was established with four highly precise assays performed in one central laboratory. Therefore, discrepancies in the MBG-HbA_{1c} relationship among individuals are less likely due to errors in the measurements of either MBG or HbA_{1c}.

Limitations

Although the ADAG study population was selected to limit factors known to interfere with the measurement of MBG or HbA_{1c}, or with the relationship between them, inter-individual differences, such as ethnicity, age and gender were of course not excluded.

Other limitations of this study include the limited range of reliable measurements outside the 40 mg/dL and 400 mg/dL (2.2 and 22.2 mmol/L) when using CGM and the variation in MBG measurement with the Lifescan meter. Although it is one of the largest studies examining the relationship between HbA_{1c} and MBG, the relatively small sample size of the subpopulations may have affected our findings. Finally, the measurement of HGI is not independent of the HbA_{1c} level, so the associations documented with HGI may be confounded by the HbA_{1c} level itself [55].

Conclusions

We concluded that higher GV was associated with higher HGI. Measures of GV (SD, MAGE and $CONGA_4$) and AUC_{24} and fructosamine are strongly correlated with HGI and high outlier status. The GV measure SD and smoking status explained the largest fraction of outlier status for the High outliers. These variables together explained only around 13 % of the variance in the MBG-HbA_{1c} relationship. Finally, diabetes type, Apo-B levels and insulin and lipid treatment were associated with High outliers. However, all these factors combined explained only 25% of the variance in the MBG-HbA_{1c} relationship for the High outliers.

1,5 AnhydroGlucitol (Chapter 5)

Markers for longer-term glycemic control, including fructosamine and HbA_{1c}, reflect average glucose concentrations over 2 and 8 till 10 weeks respectively, but do not provide information on GV. Patients in acceptable glycemic control according to HbA_{1c} levels may still have significant postprandial hyperglycemia [56].

Plasma 1,5AG is cleared renally by competitive inhibition of reabsorption at glucose levels above the renal threshold for glucose. Previous studies have shown reduced 1,5AG levels in hyperglycemic patients. Therefore, 1,5AG has been proposed as a marker of glycemic excursions. We examined whether 1,5AG might be used as an indicator of GV including overall (postprandial) hyperglycemic episodes at predefined HbA_{1c} ranges.

Conclusions

We concluded that the test performance of 1,5AG to detect hyperglycemic episodes in moderately controlled patients (HbA1c ≤ 64 mmol/mol (8%)) was fair (AUC of ROC curve 0.73, $p < 0.001$). Measures of GV and hyperglycemic episodes correlated significantly and inversely with 1,5AG at HbA_{1c} levels ≤ 64 mmol/mol (8%) and between 42 and 64 mmol/mol (6 - 8%). Measuring 1,5AG in addition to HbA_{1c} may identify GV and postprandial hyperglycemia, especially in moderately controlled patients with diabetes.

Limitations

The main limitation of this study was that all variables were measured at one single time point (one 48-hour period), which does not fully cover the time period reflected by the different measured parameters (HbA_{1c} and 1,5AG). Since, only participants in relatively stable glycemic control were included in the study, we assumed that the GV measures and MBG, assessed at this time point, were representative for the period prior to the measurement. Also, we couldn't correct 1,5AG values for kidney function, but participants with severe renal impairment were excluded.

1,5AG in routine daily practice?

1,5AG may be used as an additional tool to monitor glycemic control during the 2 to 3 weeks prior to the HbA_{1c} measurement. This may motivate patients to monitor glycemic excursions and to achieve better glycemic control. The down side is the need of an additional blood sample as, since at present, no home-test for 1,5AG is available.

The reference range of the 1,5AG test is established in persons without diabetes and is quite broad, indicating a large biological variation in the population. This, and also the fact that the 1,5AG concentration is influenced by the level of MBG and $HbA_{1c,}$ makes the test less reliable and less easy to interpret. Patients can easily check their glycemic control by performing SMBG at relevant time points, and by assessing an HbA_{1c} test every 3 months.

Unfortunately it is not useful in pregnant women, a patient group where tight glycemic control is of special importance, as the glomerular filtration rate can change during pregnancy. Abnormal values have also been noted in individuals with abnormal glomerular filtration rates [57]. Low 1,5AG values have also been observed in terminal stage renal failure, dialysis patients, advanced cirrhosis, and prolonged fasting. More studies are required to establish the clinical utility of measuring 1,5AG, and in particular in specific patient populations, as for example in pregnancy.

Associations between different glucose indices and HbA_{1c} (Chapter 6)

Assessing glucose exposure

Various established methods exist to quantify postprandial glycemia or GV, but only few have been compared with each other and with HbA_{1c}. We examined the relationship among common indices of GV, average glycemia, postprandial glycemia and HbA_{1c} using detailed glucose measures obtained during real-life in the ADAG study cohort. As we expected, our analyses revealed that many of these glycemic indices were strongly correlated within each category. Additionally, we studied which

blood glucose value(s) of the day provide the strongest prediction of MBG, as measured by HbA_{1c}, especially focusing on pre- and postprandial glucose contributions to MBG levels.

Indices of postprandial glycemia and glucose variability

Especially indices of GV (CONGA, SD of CGM or SMBG, and MAGE) were highly intercorrelated indicating that these calculated measures of variability express almost identical information. MAGE has previously been described as the 'gold standard' of assessing GV [28].

Our findings show that MAGE and CONGA or the 'simple' standard deviation (SD) capture information regarding variability to a very similar degree, indicating that the choice can be made on the basis of ease of calculation or practical considerations. The variability measures did not correlate well with the postprandial measurements or indexes of fasting or average glycemia.

The CGM captured postprandial AUC 2-hours following a meal correlates well with the SMBG postprandial measurements. This means that the glucose excursion in the hours after a meal is reliably captured by a routine 90 minutes postprandial SMBG measurement. Both the SMBG and the CGM postprandial measurements correlate moderately with overall hyperglycemia as measured with CGM (AUC >11.1 mmol/l), average BG and HbA_{1c}.

A 'postprandial increment' has been used to assess GV and PPG in previous studies [27, 58], but the definition and calculation methods have varied. When we defined the postprandial increment as the difference in glucose level from the pre-prandial glucose concentration to highest postprandial value in a 2-hour window the index showed low correlations with postprandial BG levels (ρ= 0.45-0.51) and with indices of average glycemia or hyperglycemia (ρ= 0.26-0.27). Large postprandial increments may be expected to reflect high GV; however, the correlation to the variability measures are not strong (ρ= from 0.41 (SMBG SD) to 0.54 ($CONGA_4$)). Hence, postprandial glucose increments do not seem to be a satisfactory way to assess GV.

As expected, HbA_{1c} correlated well with average blood glucose from CGM, SMBG, and the two combined. When exploring the contribution of glucose levels from SMBG at different times of the day to average glycemia, the pre-prandial glucose levels had a larger effect on HbA_{1c} than postprandial glucose levels, presumably because they resemble the 24-h glucose levels (and thus the long-term exposure to glucose) more closely. This result was the same before and after including the nocturnal blood glucose index to the regression model, which, surprisingly, only lead to a small increase in the proportion of HbA_{1c} variation explained.

The frequently cited article by Monnier et al [27] concludes that postprandial glucose levels are the dominant contributor to HbA_{1c} levels in patients with $HbA_{1c} < 8.5\%$, while fasting glucose levels were the major contributor for patients with $HbA_{1c} > 8.5\%$. The calculations underpinning this conclusion were based on AUCs derived from meal-period measurements only, thus disregarding the contribution of glucose exposure outside meal periods to HbA_{1c}.

Monnier et al [27] define postprandial glycemia as the AUC above each individual's fasting value, while pre-prandial glycemia is defined as the AUC between 6.1 mmol/l (110 mg/dl) and measured FBG for each individual. This approach introduces a bias when comparing the association between these two indexes and HbA_{1c}. Individuals with HbA_{1c} levels 8.5% will strongly tend to also have high FBG. Their postprandial AUC values will therefore be small by artifact, as only excursions above these high individual FBG values are considered postprandial glucose exposure. Simultaneously, Monnier et al.'s definition yields larger pre-prandial AUCs in this same group, thus introducing the reported effect. This methodological problem might explain why Monnier et al.'s results differ from our findings and those of others [59, 60].

The putative roles of GV and PPG as risk factors for diabetes complications are based on 1) studies reporting an association between excessive PPG levels and factors that may lead to development of diabetes complications [38, 61-63], 2) epidemiological studies associating 2-h post-OGTT values with increased mortality and cardiovascular disease [64-67], and 3) a few clinical trials in very specific subgroups (e.g. pregnant

women [68] and individuals with impaired glucose tolerance [69] or T2DM post-AMI [70]), which have addressed the issue with different methods and have had conflicting results. The roles of PPG and GV as risk markers need further exploration, and an understanding of the differences and similarities among the different measures of PPG, overall hyperglycemia, and GV is critical.

Fasting Blood Glucose (FBG) levels were only moderately correlated with indexes of hyperglycemia and average or postprandial glucose levels.

The ADAG glucose monitoring protocol was intensive and not feasible in daily clinical routine. Measurements of FBG, well timed postprandial glucose and HbA_{1c} are much easier to implement in clinical care.

CGM versus SMBG

Conventional SMBG is well known and regularly used by most patients. CGM has the advantage of a comprehensive BG data collection and has a marked educational potential, but also requires considerable additional resources, especially staff and education facilities. This makes CGM more costly to implement in daily clinical practice as well as in research settings. These extra resources might, arguably, be cost-effective when the goal is to improve overall glucose control [71] but the use of CGM does not seem to be necessary for assessing the degree of variability and PPG in situations where frequent SMBG is feasible.

Limitations

The fact that participants had stable HbA_{1c} (< 1% HbA_{1c} change 6 month prior to study) could have led to underestimation of GV. However, high levels of GV were seen among our subjects despite stable HbA_{1c} values. Even though patients with T1DM and T2DM have different glucose patterns because of different pathophysiologies, the mechanism of hemoglobin glycation is likely to be the same. The relationships of the glycemic indices were therefore calculated for the combined group.

Conclusion

The relevance of glucose excursions and postprandial glycemia in the day-to-day diabetes control and risk management is still debated. Different

indices based on different monitoring and calculation methods intercorrelate well within each category (variability, postprandial, average indices). Variability indices are weakly correlated with the other categories indicating that these measures convey different information. However, indices of postprandial, average, and overall hyperglycemia correlate moderately between categories.

Our findings confirm that FBG is not a clear indicator of general glycemia. The mean of all pre-prandial, as compared to postprandial glucose values have a stronger relationship with $HbA_{1c,}$ both in patients with T1DM and T2DM.

Glucose variability in clinical practice

Clinicians must understand GV both qualitatively and quantitatively and endeavor to reduce that variability before trying to reduce the mean level of blood glucose. This sounds intuitively obvious [72, 73] and can also be demonstrated mathematically. If the mean glucose level was 5.6 mmol/L (100 mg/dL) but the SD was 2.2 mmol/L (40 mg/dL), one could predict that there would be an unacceptable incidence of severe hypoglycemia even though the mean glucose is in the euglycemic range.

This applies to blood glucose as measured by SMBG, laboratory measurements of venous samples and interstitial glucose as measured by CGM. When titrating a medication such as basal insulin, it is essential to know the between-day (within-subject) variability in fasting plasma glucose to be able to set the target glucose level appropriately so that risk of hypoglycemia is at an acceptable level. Unfortunately, these estimates of GV are rarely obtained.

GV also serves as one facet of the quality of glycemic control, another reason to quantify GV. Epidemiologic and preclinical studies suggest that GV contributes to the risk of complications in diabetes [72, 74-81]. This hypothesis remains controversial and will remain an active area of research [82-92].

The above three considerations, the requirement to achieve good control, the desire to assess quality of glycemic control, and the plausible link to

complications, provide a major impetus for development, testing and application of methods to quantify GV. To assist the clinician with the interpretation of measures of GV, we need to have ‘‘normative’’ or ‘‘reference’’ data. Data obtained in non-diabetic individuals, as reported by Mazze et al. [93] and Zhou et al. [94], are helpful in setting a baseline. However, these values are so far removed from what is observed in patients with diabetes that they have only minimal relevance.
We need to be able to assess the observed variability in a large population (or populations) of people with diabetes (T1DM and T2DM). Because most measures of GV are closely related to mean glucose and HbA_{1c} levels, criteria should be developed for multiple ranges of HbA_{1c} values.

Several groups have developed computer programs to calculate GV. These include methods for calculation of MAGE [95, 96], software called a ‘‘Gly-Culator’’ [97] and spreadsheets to calculate various types of SDs [98, 99] among others. The aim was to introduce a degree of standardization and thereby reduce the risk of errors in the computations.

Now there is a plethora of measures of GV, and the number continues to grow [72, 74-79, 100-103]. We need to make sure that these parameters become clinically useful, by providing reference ranges for defined types of patients (defined by type of diabetes, type of therapy, degree of glycemic control by the ‘‘gold standard’’ HbA_{1c}) [104]. Data reduction needs to be fully automated, whether the glucose data are generated from SMBG, CGM, or hospital-based systems.

Real life glycemic profiles in non-diabetic individuals (Chapter 7)

Glucose profiles obtained in healthy persons under real-life conditions may serve as a benchmark for studies in patients with hyperglycemia. Current understanding of normoglycemia is largely based on studies of populations without diabetes, with a limited number of glucose measurements per individual in experimental conditions. Real-life glycemic profiles of healthy individuals are not readily available [93, 94].

In the ADAG study real-life glycemia, including PPG concentrations from 80 individuals without diabetes was obtained. The objective was to assess glycemic variability in individuals without diabetes and to study whether OGTT thresholds for impaired glucose tolerance (IGT) and diabetes were exceeded in real life. The median time of CGM (over a 3 months period) was 230 hours per individual offering detailed information on glucose features under real-life conditions and allowed several approaches to define PPG, and provided sufficient measurements to reliably assess features of GV (SD).

We found that nearly all (93%) individuals without diabetes exceeded the IGT threshold of 7.8 mmol/l at some point during the day and spent a median of 26 minutes (range 0 min – 6 h 52 min) per day above this level. Eight individuals (10%) spent more than 2 hours in the IGT range. One in ten reached levels (11.1 mmol/l) diagnostic of diabetes. These findings suggest that ambient glucose levels in persons without diabetes are frequently in the IGT range and that a substantial proportion reach even higher levels.

This highlights that, even though the monitored non-diabetic individuals in the ADAG study were selected by a very low level of baseline fasting plasma glucose (FPG), some of the exposure to moderately elevated glucose levels remains out of sight when we classify individuals based on isolated glucose measurements and HbA_{1c} levels. Previous smaller studies have suggested similar patterns, albeit in more homogeneous populations [93, 105].

Glucose and HbA_{1c} levels from persons without diabetes and patients with IGT/diabetes are part of a continuum; there are no strict cut-off points, but a gradual distribution.

During a standardized OGTT, it is well established that glucose concentrations can exceed 7.8 mmol/l in individuals with normal glucose tolerance in the time preceding the 2-hour value [106]. However, since the 75g OGTT is an extreme liquid glucose load compared to an average mixed meal, we find that our results based on real-life monitoring add an important dimension.

Limitations

A limitation of the ADAG study when examining individuals without diabetes is the absence of OGTTs at screening to rule out diabetes with certainty or to classify subjects as having IGT. However, our fasting PG exclusion criterion of < 5.4 mmol/l has been shown to be highly specific for ruling out diabetes [107].

In addition, the exclusion criterion of HbA_{1c} > 6.5% used in the ADAG study was recently proposed as the new diagnostic level for diabetes [108]. The mean HbA_{1c} for non-DM in the study was considerably lower: 5.2 % (SD 0.3). Furthermore, it would have been interesting to analyze measures of glucose fluctuations.

HbA_{1c} and mean blood glucose show stronger associations with cardiovascular disease risk factors than do postprandial glycemia or glucose variability in persons with diabetes (Chapter 8)

Assessing glucose exposure

Increased glucose excursions and postprandial hyperglycemia have been suggested as unique risk factors of cardiovascular disease (CVD) and mortality in patients with diabetes mellitus. Much of the evidence is based on a single 2-hour glucose value after oral glucose tolerance testing in epidemiological studies. Treatment regimens and guidelines have increasingly focused on PPG control as an additional target beyond average glucose control.

However, direct evidence for an additional effect of controlling PPG excursions - over and above an effect on reduced average glucose levels - on relevant diabetic endpoints is limited.

Only a few studies have tested this hypothesis directly or compared the effect with that of overall glucose exposure (HbA_{1c}) and shown PPG levels and/or GV to be independent mechanisms. One single-blind randomised trial comparing the effects of two insulin secretagogues with different

effects on PPG found that control of postprandial hyperglycemia led to a reduction in carotid intima–media thickness in patients with T2DM compared with the control group [109]. Therapy with lower PPG levels was associated with significant reductions in the concentrations of the inflammatory markers IL6 and hs-CRP. A recent randomized clinical trial in patients with T2DM and CVD did not support an added benefit of targeting control of PPG on subsequent CVD events [70]. However, in this study the difference in PPG between the 2 intervention groups might have been to small to find this effect.

We examined the association between various indices of glycemia measured during every-day activities and metabolic CVD risk factors (lipids, hs-C-reactive protein, blood pressure). In order to correlate the risk factors of CVD to glucose exposure, we had to define categories of commonly used indices of glycemic variability, average and postprandial glycemia. As we expected, our analyses revealed that many of these glycemic indices were strongly correlated within each category.

In our study, indices of GV showed no significant associations with CVD risk factors. GV and postprandial hyperglycemia were not stronger associated with known metabolic CVD risk factors than measures of average glucose. This suggests that the impact of PPG on cardiovascular risk is likely to be captured by the assessment of average blood glucose or HbA_{1c}.
Several epidemiological studies demonstrating an association between post-OGTT hyperglycemia and increased CVD and mortality, did not take an average glucose measurement (for example by HbA_{1c}) into account [64, 65, 110, 111].
Moreover, the thorough measurement of glycemia under real-life circumstances in the ADAG study provides a more reliable index of day-to-day exposure than the usual single measurement of glucose levels after an OGTT.

In addition, the intensive glucose monitoring with several methods allowed several approaches to define PPG, and provided sufficient measurements to assess reliably the different features of glycemia such as GV.

In T1DM, GV has not been shown to be associated with the development of complications. In the DCCT, GV (from seven- point profiles) did not appear to be a factor in the development of micro-vascular complications, and pre- and postprandial glucose values contributed equally to small-vessel complications [84].

The CVD risk factors we chose are well-validated or "traditional" risk factors of CVD (lipids [112-114] and blood pressure [115]) and one indicator of low-grade inflammation (hs-CRP) [116]. We have considered the possible impact of treatment to lower these risk factors on our findings by excluding participants receiving lipid lowering or anti-hypertension treatment. This did not substantially alter the results.

The associations between the calculated glycemic indices and CVD risk factors were explored in individual linear regression models adjusted for age, gender, and diabetes type. We considered the use of multivariate models including both average and postprandial glycemia. We decided against this model as average blood glucose and HbA_{1c} are closely related to the meal-related glucose values, and such analyses would allow small fluctuations to be highly influential.

To facilitate comparison of associations, potentially explanatory glycemic variables were standardized by the study population standard deviation (SD). As our data are cross-sectional and without information on CVD outcomes, we considered ways to estimate risk of CVD as a continuous outcome. A well-established, reproducible risk score like the UKPDS risk score would have been a way to do this. However, since no data regarding atrial fibrillation and diabetes duration were available, both of which factors are included in the UKPDS risk engine, this risk analysis tool could not be used. Therefore, a combined Z-score was calculated from the standardized CVD risk factors. This Z-score is based on the distribution in the present study population (standardized by SD), and thus results are not comparable to other populations. However, using this score gave us an index for a combined cardiovascular risk for each individual.

Our results do not support a unique role of postprandial hyperglycemia in CVD. Monitoring PPG and GV may be important in adjusting treatment to

achieve target mean glycemia and to avoid daily excursions including hypoglycemia, but our results suggest that interventions to reduce CVD risk are best aimed at controlling mean glucose and HbA_{1c}.

Limitations

The main limitation of the study in this context is it's cross sectional character. While it has a very high resolution, the glucose monitoring is short term and our outcomes are CVD risk factors rather than actual CVD events. Therefore, this study cannot reach direct conclusions regarding the impact of PPG levels or GV on CVD endpoints. However, our results indicate that if such an effect exists, it is unlikely to be mediated through the mechanisms (risk factors) examined in our study.

Furthermore, the participants had stable HbA_{1c} at baseline (defined as a < 1 % unit change in HbA_{1c} during the 6 months prior to the study), and were relatively stable during the study. We may therefore have limited the range of GV as seen in a diabetic population. However, high levels of GV were seen among our individuals despite stable HbA_{1c} levels.

Conclusions

Mean glycemia and HbA_{1c} show consistent associations with CVD risk factors at a stronger level than fasting glucose and most measures of PPG and GV. In our study, the previously observed associations between GV and PPG and CVD events cannot be explained by an association with known metabolic CVD risk factors.

Future perspectives

The NGSP certification process will ensure standardization and the IFCC Laboratory Network will continue to serve as a second anchor for the NGSP. The reporting of HbA_{1c} test results to clinicians, patients and in the scientific literature will however vary across countries and regions. Scientific reporting will gradually change to the SI units (mmol/mol), whereas physicians and patients will continue to use DCCT or estimated Average Glucose values.

HbA_{1c} will increasingly be used for diagnostic purposes and in primary care and in emerging countries. This increased demand has led to a greater supply for new and cheaper HbA_{1c} assay systems. The higher cost, compared to the glucose assay, is set-off by ease of use (no fasting required) and potentially less personnel as glucose tolerance tests will not be required.

Glucose variability will continue to capture the interest of diabetes researchers. The questions that need to be addressed include: what is the best measure of glucose variability in daily life? How to define it for different patient groups and for different levels of glycemic control? How to implement this in daily clinical practice? New technology for continuous glucose monitoring has led to the availability of large numbers of blood glucose measurements. Now we need the software to develop clinically meaningful, i.e. actionable information from these rich data sources.

Another area of interest remains the role of glucose variability in oxidative stress, and the putative relationship with the development of diabetes related complications. This needs to include a better understanding of the role of oxidative stress and advanced glycation end products (AGE) in the pathophysiology of complications. The focus will move away from postprandial glucose to all aspects of glucose variability.

1.5AG has been propagated as clinically meaningful information for patients. New assay systems will be developed for home-use. This will hopefully support patients to reach and keep good and more stable glycemic control. This needs to be established in well-controlled studies.

Conclusions

The overall conclusions of this Ph.D. thesis are:

The ADAG study showed a simple linear relationship between mean glucose and HbA_{1c} levels in a clinically relevant range of glycemia for patients with T1DM and T2DM. Factors influencing this relationship are; race/ethnicity, smoking, high glucose variability, altered erythropoiesis, altered erythrocyte lifespan, pregnancy, renal failure, bleeding, blood transfusion and hemoglobinopathies.

HbA_{1c} can be translated into an eAG with a standard deviation of 0.87 mmol/l. The worldwide use of eAG in clinical practice has failed.

The worldwide standardization on how to report HbA_{1c} test results has not been successful. The comparability of assay's worldwide has improved but the reporting and interpretation of
HbA_{1c} in clinical data and research results in the diabetes field worldwide has become more complicated.

We found that all GV measures modestly, but significantly, influenced the MBG-HbA_{1c} relationship.

Higher GV was associated with higher HGI. Measures of GV (SD, MAGE and $CONGA_4$) and fructosamine are strongly correlated with HGI and high outlier status.

The GV measure SD and smoking status explained the largest fraction of outlier status for the High outliers. These variables together explained only around 13 % of the variance in the MPG-HbA_{1c} relationship.

Measuring 1,5AG in addition to HbA_{1c} may identify GV and postprandial hyperglycemia, especially in moderately controlled patients with diabetes.

In general, calculations based on CGM were not more informative than those based on frequent 7-point SMBG.

Indices of variability did not correlate strongly with indices of fasting, postprandial or total hyperglycemia.

The mean of all pre-prandial glucose levels had a larger impact on HbA_{1c} levels than postprandial glucose levels in patients with T1DM and T2DM.

Non-diabetic individuals under real-life conditions spent a considerable amount of time with blood glucose levels classified as ‘pre-diabetic’ or even diabetic.

Mean glycemia and HbA_{1c} show stronger and more consistent associations with CVD risk factors than fasting glucose or postprandial glucose levels or measures of GV in patients with diabetes.

REFERENCE LIST

1. Nathan, D.M., et al., Translating the A1C assay into estimated average glucose values. Diabetes Care, 2008. 31(8): p. 1473-8.
2. Rohlfing, C.L., et al., Defining the relationship between plasma glucose and HbA(1c): analysis of glucose profiles and HbA(1c) in the Diabetes Control and Complications Trial. Diabetes Care, 2002. 25(2): p. 275-8.
3. Nathan, D.M., H. Turgeon, and S. Regan, Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia, 2007. 50(11): p. 2239-44.
4. Pani, L.N., et al., Effect of aging on A1C levels in individuals without diabetes: evidence from the Framingham Offspring Study and the National Health and Nutrition Examination Survey 2001-2004. Diabetes Care, 2008. 31(10): p. 1991-6.
5. Davidson, M.B. and D.L. Schriger, Effect of age and race/ethnicity on HbA1c levels in people without known diabetes mellitus: implications for the diagnosis of diabetes. Diabetes Res Clin Pract, 2010. 87(3): p. 415-21.
6. Kilpatrick, E.S., M.H. Dominiczak, and M. Small, The effects of ageing on glycation and the interpretation of glycaemic control in Type 2 diabetes. Qjm, 1996. 89(4): p. 307-12.
7. Gulliford, M.C. and O.C. Ukoumunne, Determinants of glycated haemoglobin in the general population: associations with diet, alcohol and cigarette smoking. Eur J Clin Nutr, 2001. 55(7): p. 615-23.
8. Faerch, K., et al., Sex differences in glucose levels: a consequence of physiology or methodological convenience? The Inter99 study. Diabetologia, 2010. 53(5): p. 858-65.
9. Simon, D., et al., Epidemiological features of glycated haemoglobin A1c-distribution in a healthy population. The Telecom Study. Diabetologia, 1989. 32(12): p. 864-9.
10. Modan, M., et al., Significance of high HbA1 levels in normal glucose tolerance. Diabetes Care, 1988. 11(5): p. 422-8.
11. Bleyer, A.J., et al., Ethnic variation in the correlation between random serum glucose concentration and glycated haemoglobin. Diabet Med, 2009. 26(2): p. 128-33.
12. Herman, W.H. and R.M. Cohen, Racial and ethnic differences in the relationship between HbA1c and blood glucose: implications for the diagnosis of diabetes. J Clin Endocrinol Metab, 2012. 97(4): p. 1067-72.
13. Herman, W.H., et al., Differences in A1C by race and ethnicity among patients with impaired glucose tolerance in the Diabetes Prevention Program. Diabetes Care, 2007. 30(10): p. 2453-7.
14. Ziemer, D.C., et al., Glucose-independent, black-white differences in hemoglobin A1c levels: a cross-sectional analysis of 2 studies. Ann Intern Med, 2010. 152(12): p. 770-7.

15. Likhari, T. and R. Gama, Ethnic differences in glycated haemoglobin between white subjects and those of South Asian origin with normal glucose tolerance. J Clin Pathol, 2010. 63(3): p. 278-80.
16. Herman, W.H., et al., Racial and ethnic differences in mean plasma glucose, hemoglobin A1c, and 1,5-anhydroglucitol in over 2000 patients with type 2 diabetes. J Clin Endocrinol Metab, 2009. 94(5): p. 1689-94.
17. Boeing, H., et al., Association between glycated hemoglobin and diet and other lifestyle factors in a nondiabetic population: cross-sectional evaluation of data from the Potsdam cohort of the European Prospective Investigation into Cancer and Nutrition Study. Am J Clin Nutr, 2000. 71(5): p. 1115-22.
18. Harding, A.H., et al., Cross-sectional association between total level and type of alcohol consumption and glycosylated haemoglobin level: the EPIC-Norfolk Study. Eur J Clin Nutr, 2002. 56(9): p. 882-90.
19. Meyer, K.A., et al., Alcohol consumption patterns and HbA1c, C-peptide and insulin concentrations in men. J Am Coll Nutr, 2003. 22(3): p. 185-94.
20. Higgins, T., et al., Influence of variables on hemoglobin A1c values and nonheterogeneity of hemoglobin A1c reference ranges. J Diabetes Sci Technol, 2009. 3(4): p. 644-8.
21. Sargeant, L.A., et al., Cigarette smoking and glycaemia: the EPIC-Norfolk Study. European Prospective Investigation into Cancer. Int J Epidemiol, 2001. 30(3): p. 547-54.
22. Koga, M., et al., Serum glycated albumin levels are influenced by smoking status, independent of plasma glucose levels. Acta Diabetol, 2009. 46(2): p. 141-4.
23. Cerami, C., et al., Tobacco smoke is a source of toxic reactive glycation products. Proc Natl Acad Sci U S A, 1997. 94(25): p. 13915-20.
24. Sagone, A.L., Jr., T. Lawrence, and S.P. Balcerzak, Effect of smoking on tissue oxygen supply. Blood, 1973. 41(6): p. 845-51.
25. Smith, R.J., et al., Regulation of hemoglobin AIc formation in human erythrocytes in vitro. Effects of physiologic factors other than glucose. J Clin Invest, 1982. 69(5): p. 1164-8.
26. Consensus, C., Consensus Statement on the Worldwide Standardization of the Hemoglobin A1C Measurement: The American Diabetes Association, European Association for the Study of Diabetes, International Federation of Clinical Chemistry and Laboratory Medicine, and the International Diabetes Federation, 2007. p. 2399-2400.
27. Monnier, L., H. Lapinski, and C. Colette, Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA(1c). Diabetes Care, 2003. 26(3): p. 881-5.

28. Service, F.J., et al., Mean amplitude of glycemic excursions, a measure of diabetic instability. Diabetes, 1970. 19(9): p. 644-55.
29. McDonnell, C.M., et al., A novel approach to continuous glucose analysis utilizing glycemic variation. Diabetes Technol Ther, 2005. 7(2): p. 253-63.
30. Wilson, D.M. and Kollman, Relationship of A1C to glucose concentrations in children with type 1 diabetes: assessments by high-frequency glucose determinations by sensors. Diabetes Care, 2008. 31(3): p. 381-5.
31. Derr, R., et al., Is HbA(1c) affected by glycemic instability? Diabetes Care, 2003. 26(10): p. 2728-33.
32. Service, F.J. and P.C. O'Brien, Influence of glycemic variables on hemoglobin A1c. Endocr Pract, 2007. 13(4): p. 350-4.
33. McCarter, R.J., J.M. Hempe, and S.A. Chalew, Mean blood glucose and biological variation have greater influence on HbA1c levels than glucose instability: an analysis of data from the Diabetes Control and Complications Trial. Diabetes Care, 2006. 29(2): p. 352-5.
34. Selvaraj, N., Z. Bobby, and V. Sathiyapriya, Effect of lipid peroxides and antioxidants on glycation of hemoglobin: an in vitro study on human erythrocytes. Clin Chim Acta, 2006. 366(1-2): p. 190-5.
35. Selvaraj, N., Z. Bobby, and M.G. Sridhar, Oxidative stress: does it play a role in the genesis of early glycated proteins? Medical Hypotheses, 2008. 70(2): p. 265-8.
36. Brownlee, M., A radical explanation for glucose-induced beta cell dysfunction. J Clin Invest, 2003. 112(12): p. 1788-90.
37. Brownlee, M., The pathobiology of diabetic complications: a unifying mechanism. Diabetes, 2005. 54(6): p. 1615-25.
38. Monnier, L., et al., Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. Jama, 2006. 295(14): p. 1681-7.
39. Wentholt, I.M., et al., Glucose fluctuations and activation of oxidative stress in patients with type 1 diabetes. Diabetologia, 2008. 51(1): p. 183-90.
40. Wright, R.J. and B.M. Frier, Vascular disease and diabetes: is hypoglycaemia an aggravating factor? Diabetes Metab Res Rev, 2008. 24(5): p. 353-63.
41. Singh, P., A. Jain, and G. Kaur, Impact of hypoglycemia and diabetes on CNS: correlation of mitochondrial oxidative stress with DNA damage. Mol Cell Biochem, 2004. 260(1-2): p. 153-9.
42. Gogitidze Joy, N., et al., Effects of acute hypoglycemia on inflammatory and pro-atherothrombotic biomarkers in individuals with type 1 diabetes and healthy individuals. Diabetes Care, 2010. 33(7): p. 1529-35.
43. Wang, J., et al., Acute exposure to low glucose rapidly induces endothelial dysfunction and mitochondrial oxidative stress: role

for AMP kinase. Arterioscler Thromb Vasc Biol, 2012. 32(3): p. 712-20.
44. Giacco, F. and M. Brownlee, Oxidative stress and diabetic complications. Circ Res, 2010. 107(9): p. 1058-70.
45. Ceriello, A. and M. Ihnat, Oxidative stress is, convincingly, the mediator of the dangerous effects of glucose variability. Diabet Med, 2010. 27(8): p. 968.
46. Ceriello, A., Hyperglycaemia and the vessel wall: the pathophysiological aspects on the atherosclerotic burden in patients with diabetes. Eur J Cardiovasc Prev Rehabil, 2010. 17 Suppl 1: p. S15-9.
47. Nandish, S., et al., Implementing cardiovascular risk reduction in patients with cardiovascular disease and diabetes mellitus. Am J Cardiol, 2011. 108(3 Suppl): p. 42B-51B.
48. Ceriello, A., et al., Evidence that hyperglycemia after recovery from hypoglycemia worsens endothelial function and increases oxidative stress and inflammation in healthy control subjects and subjects with type 1 diabetes. Diabetes, 2012. 61(11): p. 2993-7.
49. Kilpatrick, E.S., P.W. Maylor, and B.G. Keevil, Biological variation of glycated hemoglobin. Implications for diabetes screening and monitoring. Diabetes Care, 1998. 21(2): p. 261-4.
50. Hempe, J.M., et al., High and low hemoglobin glycation phenotypes in type 1 diabetes: a challenge for interpretation of glycemic control. J Diabetes Complications, 2002. 16(5): p. 313-20.
51. Gould, B.J., S.J. Davie, and J.S. Yudkin, Investigation of the mechanism underlying the variability of glycated haemoglobin in non-diabetic subjects not related to glycaemia. Clin Chim Acta, 1997. 260(1): p. 49-64.
52. Yudkin, J.S., et al., Unexplained variability of glycated haemoglobin in non-diabetic subjects not related to glycaemia. Diabetologia, 1990. 33(4): p. 208-15.
53. Hudson, P.R., et al., Differences in rates of glycation (glycation index) may significantly affect individual HbA1c results in type 1 diabetes. Ann Clin Biochem, 1999. 36 (Pt 4): p. 451-9.
54. Madsen, H., J.J. Kjaergaard, and J. Ditzel, Relationship between glycosylation of haemoglobin and the duration of diabetes: a study during the third trimester of pregnancy. Diabetologia, 1982. 22(1): p. 37-40.
55. Sacks, D.B., D.M. Nathan, and J.M. Lachin, Gaps in the glycation gap hypothesis. Clin Chem, 2011. 57(2): p. 150-2.
56. Erlinger, T.P. and F.L. Brancati, Postchallenge hyperglycemia in a national sample of U.S. adults with type 2 diabetes. Diabetes Care, 2001. 24(10): p. 1734-8.
57. Kilpatrick, E.S., et al., Plasma 1,5-anhydroglucitol concentrations are influenced by variations in the renal threshold for glucose. Diabet Med, 1999. 16(6): p. 496-9.

58. Fiallo-Scharer, R., Eight-point glucose testing versus the continuous glucose monitoring system in evaluation of glycemic control in type 1 diabetes. J Clin Endocrinol Metab, 2005. 90(6): p. 3387-91.
59. Hillman, N., et al., What is the relative contribution of blood glucose levels at different time points of the day to HbA1c in Type 1 diabetes? Diabet Med, 2004. 21(5): p. 468-70.
60. Bonora, E., et al., Plasma glucose levels throughout the day and HbA(1c) interrelationships in type 2 diabetes: implications for treatment and monitoring of metabolic control. Diabetes Care, 2001. 24(12): p. 2023-9.
61. Ceriello, A., The post-prandial state and cardiovascular disease: relevance to diabetes mellitus. Diabetes Metab Res Rev, 2000. 16(2): p. 125-32.
62. Lefebvre, P.J. and A.J. Scheen, The postprandial state and risk of cardiovascular disease. Diabet Med, 1998. 15 Suppl 4: p. S63-8.
63. Heine, R.J. and J.M. Dekker, Beyond postprandial hyperglycaemia: metabolic factors associated with cardiovascular disease. Diabetologia, 2002. 45(4): p. 461-75.
64. Glucose tolerance and mortality: comparison of WHO and American Diabetes Association diagnostic criteria. The DECODE study group. European Diabetes Epidemiology Group. Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe. Lancet, 1999. 354(9179): p. 617-21.
65. Hanefeld, M., et al., Risk factors for myocardial infarction and death in newly detected NIDDM: the Diabetes Intervention Study, 11-year follow-up. Diabetologia, 1996. 39(12): p. 1577-83.
66. de Vegt, F., et al., Hyperglycaemia is associated with all-cause and cardiovascular mortality in the Hoorn population: the Hoorn Study. Diabetologia, 1999. 42(8): p. 926-31.
67. Balkau, B., et al., High blood glucose concentration is a risk factor for mortality in middle-aged nondiabetic men. 20-year follow-up in the Whitehall Study, the Paris Prospective Study, and the Helsinki Policemen Study. Diabetes Care, 1998. 21(3): p. 360-7.
68. de Veciana, M., et al., Postprandial versus preprandial blood glucose monitoring in women with gestational diabetes mellitus requiring insulin therapy. N Engl J Med, 1995. 333(19): p. 1237-41.
69. Chiasson, J.L., et al., Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. Jama, 2003. 290(4): p. 486-94.
70. Raz, I., et al., Effects of prandial versus fasting glycemia on cardiovascular outcomes in type 2 diabetes: the HEART2D trial. Diabetes Care, 2009. 32(3): p. 381-6.
71. Tamborlane, W.V., et al., Continuous glucose monitoring and intensive treatment of type 1 diabetes. N Engl J Med, 2008. 359(14): p. 1464-76.

72. Cameron, F.J., P.A. Baghurst, and D. Rodbard, Assessing glycemic variation: why, when and how? Pediatr Endocrinol Rev, 2010. 7 Suppl 3: p. 432-44.
73. Rodbard, D., Optimizing display, analysis, interpretation and utility of self-monitoring of blood glucose (SMBG) data for management of patients with diabetes. J Diabetes Sci Technol, 2007. 1(1): p. 62-71.
74. Kilpatrick, E.S., Arguments for and against the role of glucose variability in the development of diabetes complications. J Diabetes Sci Technol, 2009. 3(4): p. 649-55.
75. Weber, C. and O. Schnell, The assessment of glycemic variability and its impact on diabetes-related complications: an overview. Diabetes Technol Ther, 2009. 11(10): p. 623-33.
76. Hirsch, I.B., Glycemic variability: it's not just about A1C anymore! Diabetes Technol Ther, 2005. 7(5): p. 780-3.
77. Hirsch, I.B. and M. Brownlee, The effect of glucose variability on the risk of microvascular complications in type 1 diabetes. Diabetes Care, 2007. 30(1): p. 186-7; author reply 188-9.
78. Brownlee, M. and I.B. Hirsch, Glycemic variability: a hemoglobin A1c-independent risk factor for diabetic complications. Jama, 2006. 295(14): p. 1707-8.
79. Schisano, B., et al., Glucose oscillations, more than constant high glucose, induce p53 activation and a metabolic memory in human endothelial cells. Diabetologia, 2011. 54(5): p. 1219-26.
80. Siegelaar, S.E., et al., Glucose variability; does it matter? Endocr Rev, 2010. 31(2): p. 171-82.
81. Muggeo, M., et al., Fasting plasma glucose variability predicts 10-year survival of type 2 diabetic patients: the Verona Diabetes Study. Diabetes Care, 2000. 23(1): p. 45-50.
82. Siegelaar, S.E., et al., Glucose variability does not contribute to the development of peripheral and autonomic neuropathy in type 1 diabetes: data from the DCCT. Diabetologia, 2009. 52(10): p. 2229-32.
83. Siegelaar, S.E., et al., A randomized clinical trial comparing the effect of basal insulin and inhaled mealtime insulin on glucose variability and oxidative stress. Diabetes Obes Metab, 2009. 11(7): p. 709-14.
84. Kilpatrick, E.S., A.S. Rigby, and S.L. Atkin, The effect of glucose variability on the risk of microvascular complications in type 1 diabetes. Diabetes Care, 2006. 29(7): p. 1486-90.
85. Kilpatrick, E.S., A.S. Rigby, and S.L. Atkin, Effect of glucose variability on the long-term risk of microvascular complications in type 1 diabetes. Diabetes Care, 2009. 32(10): p. 1901-3.
86. Bragd, J., et al., Can glycaemic variability, as calculated from blood glucose self-monitoring, predict the development of complications in type 1 diabetes over a decade? Diabetes Metab, 2008. 34(6 Pt 1): p. 612-6.

87. Snell-Bergeon, J.K., et al., Glycaemic variability is associated with coronary artery calcium in men with Type 1 diabetes: the Coronary Artery Calcification in Type 1 Diabetes study. Diabet Med, 2010. 27(12): p. 1436-42.
88. Siegelaar, S.E., et al., A decrease in glucose variability does not reduce cardiovascular event rates in type 2 diabetic patients after acute myocardial infarction: a reanalysis of the HEART2D study. Diabetes Care, 2011. 34(4): p. 855-7.
89. Monnier, L. and C. Colette, Glycemic Variability: Should we and can we prevent it? Diabetes Care, 2008. 31(Supplement_2): p. S150-154.
90. Monnier, L. and C. Colette, Glycemic variability: can we bridge the divide between controversies? Diabetes Care, 2011. 34(4): p. 1058-9.
91. Borg, R., et al., HbA(1)(c) and mean blood glucose show stronger associations with cardiovascular disease risk factors than do postprandial glycaemia or glucose variability in persons with diabetes: the A1C-Derived Average Glucose (ADAG) study. Diabetologia, 2011. 54(1): p. 69-72.
92. Standl, E., O. Schnell, and A. Ceriello, Postprandial hyperglycemia and glycemic variability: should we care? Diabetes Care, 2011. 34 Suppl 2: p. S120-7.
93. Mazze, R.S., et al., Characterizing glucose exposure for individuals with normal glucose tolerance using continuous glucose monitoring and ambulatory glucose profile analysis. Diabetes Technol Ther, 2008. 10(3): p. 149-59.
94. Zhou, J., et al., Establishment of normal reference ranges for glycemic variability in Chinese subjects using continuous glucose monitoring. Med Sci Monit, 2011. 17(1): p. CR9-13.
95. Baghurst, P.A., Calculating the mean amplitude of glycemic excursion from continuous glucose monitoring data: an automated algorithm. Diabetes Technol Ther, 2011. 13(3): p. 296-302.
96. Fritzsche, G., et al., The use of a computer program to calculate the mean amplitude of glycemic excursions. Diabetes Technol Ther, 2011. 13(3): p. 319-25.
97. Czerwoniuk, D., et al., GlyCulator: a glycemic variability calculation tool for continuous glucose monitoring data. J Diabetes Sci Technol, 2011. 5(2): p. 447-51.
98. Rodbard, D., et al., Improved quality of glycemic control and reduced glycemic variability with use of continuous glucose monitoring. Diabetes Technol Ther, 2009. 11(11): p. 717-23.
99. Rodbard, D., L. Jovanovic, and S.K. Garg, Responses to continuous glucose monitoring in subjects with type 1 diabetes using continuous subcutaneous insulin infusion or multiple daily injections. Diabetes Technol Ther, 2009. 11(12): p. 757-65.
100. Monnier, L., et al., The contribution of glucose variability to asymptomatic hypoglycemia in persons with type 2 diabetes. Diabetes Technol Ther, 2011. 13(8): p. 813-8.

101. Dalfra, M.G., et al., Glucose variability in diabetic pregnancy. Diabetes Technol Ther, 2011. 13(8): p. 853-9.
102. Hill, N.R., et al., Normal reference range for mean tissue glucose and glycemic variability derived from continuous glucose monitoring for subjects without diabetes in different ethnic groups. Diabetes Technol Ther, 2011. 13(9): p. 921-8.
103. Marling, C.R., et al., Characterizing blood glucose variability using new metrics with continuous glucose monitoring data. J Diabetes Sci Technol, 2011. 5(4): p. 871-8.
104. Rodbard, D., Clinical interpretation of indices of quality of glycemic control and glycemic variability. Postgrad Med, 2011. 123(4): p. 107-18.
105. Derosa, G., et al., Continuous glucose monitoring system in free-living healthy subjects: results from a pilot study. Diabetes Technol Ther, 2009. 11(3): p. 159-69.
106. Abdul-Ghani, M.A., D. Tripathy, and R.A. DeFronzo, Contributions of beta-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. Diabetes Care, 2006. 29(5): p. 1130-9.
107. Genuth, S., et al., Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care, 2003. 26(11): p. 3160-7.
108. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care, 2009. 32(7): p. 1327-34.
109. Esposito, K., et al., Regression of carotid atherosclerosis by control of postprandial hyperglycemia in type 2 diabetes mellitus. Circulation, 2004. 110(2): p. 214-9.
110. Barrett-Connor, E. and A. Ferrara, Isolated postchallenge hyperglycemia and the risk of fatal cardiovascular disease in older women and men. The Rancho Bernardo Study. Diabetes Care, 1998. 21(8): p. 1236-9.
111. Shaw, J.E., et al., Isolated post-challenge hyperglycaemia confirmed as a risk factor for mortality. Diabetologia, 1999. 42(9): p. 1050-4.
112. Grundy, S.M., et al., Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. J Am Coll Cardiol, 2004. 44(3): p. 720-32.
113. Scheffer, P.G., T. Teerlink, and R.J. Heine, Clinical significance of the physicochemical properties of LDL in type 2 diabetes. Diabetologia, 2005. 48(5): p. 808-16.
114. Walldius, G. and I. Jungner, The apoB/apoA-I ratio: a strong, new risk factor for cardiovascular disease and a target for lipid-lowering therapy--a review of the evidence. J Intern Med, 2006. 259(5): p. 493-519.
115. Chobanian, A.V., et al., The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. Jama, 2003. 289(19): p. 2560-72.

116. Pearson, T.A., et al., Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation, 2003. 107(3): p. 499-511.