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Does Glucose Variability Influence the Relationship Between Mean Plasma Glucose and HbA$_{1c}$ Levels in Type 1 and Type 2 Diabetic Patients?

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OBJECTIVE—The A1C-Derived Average Glucose (ADAG) study demonstrated a linear relationship between HbA$_{1c}$ and mean plasma glucose (MPG). As glucose variability (GV) may contribute to glycation, we examined the association of several glucose variability indices and the MPG-HbA$_{1c}$ relationship.

RESEARCH DESIGN AND METHODS—Analyses included 268 patients with type 1 diabetes and 159 with type 2 diabetes. MPG during 3 months was calculated from 7-point self-monitored plasma glucose and continuous glucose monitoring. We calculated three different measures of GV and used a multiple-step regression model to determine the contribution of the respective GV measures to the MPG-HbA$_{1c}$ relationship.

RESULTS—GV, as reflected by SD and continuous overlapping net glycemic action, had a significant effect on the MPG-HbA$_{1c}$ relationship in type 1 diabetic patients so that high GV led to a higher HbA$_{1c}$ level for the same MPG. In type 1 diabetes, the impact of confounding and effect modification of a low versus high SD at an MPG level of 160 mg/dL on the HbA$_{1c}$ level is 7.02 vs. 7.43 and 6.96 vs. 7.41. All GV measures showed the same tendency.

CONCLUSIONS—In only type 1 diabetic patients, GV shows a significant interaction with MPG in the association with HbA$_{1c}$. This effect is more pronounced at higher HbA$_{1c}$ levels. However, the impact of GV on the HbA$_{1c}$ level in type 1 diabetes is modest, particularly when HbA$_{1c}$ is close to the treatment target of 7%.

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S ince the Diabetes Control and Complications Trial (DCCT) and the UK Prospective Diabetes Study (UKPDS) (1,2) established the relationship between HbA$_{1c}$ and the development of long-term diabetes complications, HbA$_{1c}$ has become the key monitoring tool in diabetes management.

During the lifetime of the erythrocyte, hemoglobin (Hb) is gradually glycated. The proportion of the glycated sites, HbA$_{1c}$, within the erythrocyte increases throughout its life span and reflect the exposure to mean blood glucose (MBG) levels during the preceding 2–3 months (3). This nonenzymatic posttranslational modification is relatively slow. In vivo and in vitro studies have shown that HbA$_{1c}$ levels are directly proportional to the time-averaged concentration of glucose during the erythrocyte's life span (3–6). Given the kinetics of glycation, brief periods of hyperglycemia should not have a major impact on HbA$_{1c}$ levels (7–9).

However, increased glycated protein levels are documented in some nondiabetic pathological states. So, hyperglycemia is not the complete answer to the etiology of increased early glycated products in nondiabetic conditions. A common denominator is oxidative stress. It has been hypothesized that oxidative stress either via increasing reactive oxygen species or by depleting the antioxidants may modulate the genesis of early glycated proteins in vivo (10,11). Hyperglycemia stimulates oxidative stress (12) and glucose variability; in particular, postprandial glucose excursions have been regarded as potentially deleterious as a result of, among other factors, their association with the increase of oxidative stress (13). Therefore, glucose variability (GV) could influence the glycation of HbA$_{1c}$.

Previous studies have examined whether the relationship between mean plasma glucose (MPG) levels and HbA$_{1c}$ is influenced by glucose variability and found no or minimal influence (10,14,15). However, these studies used limited self-monitoring of blood glucose (SMBG) data to assess mean glucose levels and variability in relatively small numbers of measurements. These methods could underestimate glycemic excursions. Continuous glucose monitoring (CGM) provides a more complete view of glycemic excursions, including the duration and frequency of the excursions, and allows calculation of features of GV. Our aim was to examine the influence of GV on the MPG-HbA$_{1c}$ relationship in the A1C-Derived Average Glucose (ADAG) study.

RESEARCH DESIGN AND METHODS—The ADAG study was conducted at 10 centers in the U.S.,

Europe, and Africa from 2006 to 2008 to define the relationship between HbA$_{1c}$ and average glucose levels. Because a full description of this observational study has been published (14), we describe it here only briefly. A total of 268 individuals with type 1 diabetes and 159 individuals with type 2 diabetes (age 18–70 years) completed the study. Participants were selected based on stable glycemic control as evidenced by two HbA$_{1c}$ values within one percentage point of each other in the 6 months prior to recruitment. Individuals with a wide range of HbA$_{1c}$ levels were included.

Participants with conditions leading to major changes in glycemia (infectious disease, steroid therapy, and pregnancy) or conditions that might interfere with the measurement of HbA$_{1c}$ or the relationship between HbA$_{1c}$ and MPG (hemoglobinopathies [16], anemia, increased erythrocyte turnover, blood loss and/or transfusions, or chronic renal or liver disease) were excluded (14). The study was approved by the human studies committees at the participating institutions, and informed consent was obtained from all participants.

Measurements of glycemia

During the study period, CGM (Medtronic Minimed, Northridge, CA) was performed at home four times with 4-week intervals during the 16-week study period. Monitoring period lasted at least 48 h, during which time glucose levels were assessed every 5 min. CGM data were accepted for analysis if there were no gaps longer than 120 min and if the mean absolute difference with the Hemocue calibration results was <18%, as recommended by the manufacturer. For calibration purposes, participants performed SMBG with the Hemocue meter (Hemocue Glucose 201 plus; Hemocue, Ängelholm, Sweden) during the days of CGM.

For adequate calculation of MPG, subjects additionally performed a seven-point SMBG (OneTouch Ultra; Lifescan, Milipitas, CA) for at least 3 days per week during the weeks when CGM was not performed. All blood glucose values stated are plasma equivalents.

HbA$_{1c}$ samples were analyzed with four highly intercorrelated DCCT-aligned assays: a high-performance liquid chromatography assay, two immunoassays, and an affinity assay (all approved by the National Glycohemoglobin Study Program). The mean HbA$_{1c}$ value at the end of the 12 week study period was used (14).

Calculating glucose variability

Three indices of intraday glucose variability were calculated based on CGM: the SD of mean glucose concentrations, the mean amplitude of glycemic excursions (MAGE), and the continuous overlapping net glycemic action (CONGA). High SD, MAGE, and CONGA values indicate high intraday glucose variability. MAGE is the mean of the differences between consecutive peaks and nadirs, only including changes of >1 SD of glycemic values and thus capturing only major fluctuations (17). For the calculation of CONGA$_n$, the difference of the current value compared with the value n hours previously was calculated for each observation after the first n hours. The CONGA$_n$ is the SD of these differences (18). In the analyses, we used CONGA at 4 h (CONGA$_4$). Calculations based on CGM data were calculated after exclusion of the initial 2 h of monitoring, which is considered to be an unstable calibration period.

Statistical analysis

First, we explored the correlations between MPG and HbA$_{1c}$ and measures of glycemic variability as SD, MAGE, and/or CONGA$_4$ for the total diabetic population and the two diabetes types. Multiple linear regression was used to investigate confounding and effect-modifying influence of clinical parameters (glycemic variability) on the relation between the determinant (MPG) and outcome (HbA$_{1c}$) of interest. We then assessed which of the variability measures (SD, MAGE, or CONGA$_4$) had the strongest impact on the MPG-HbA$_{1c}$ relationship by confounding or effect modification.

Effect modification was concluded when the slope of the interaction term of glycemic variability and determinant was significant. If no effect modification might be concluded, a parameter ΔB was computed as the relative difference of the slope of the determinant in the model without and with the clinical parameter. Confounding was concluded when the absolute value of ΔB exceeded the generally accepted threshold of 10%.

Multivariate confounding was investigated with a variant of stepwise regression, in which the stepping criterion was not a P value but the ΔB as long as it exceeded the threshold. For significance, a threshold of α = 0.05 was used.

Analyses were done for the total population and stratified for the type of diabetes. Finally, we illustrated the magnitude of the effect caused by the variability indices, by confounding or effect modification, on the MPG-HbA$_{1c}$ relationship.

RESULTS—Of the 507 patients enrolled, 427 completed the study and had adequate glucose monitoring and HbA$_{1c}$ samples for inclusion in the analyses. Two hundred and sixty-eight participants had type 1 diabetes, and 159 had type 2 diabetes. The CGM and the SMBG data during the 3-month period included approximately 2,400 and 300 measurements per subject, respectively. The relationship between the HbA$_{1c}$ level at the end of the 3-month study period and MPG calculated over the preceding 3 months was expressed as the simple linear regressions. The formula for the total diabetic population was as follows: HbA$_{1c}$ (%) = 0.028 × MPG (mg/dL) + 2.66 (R^2 = 0.80). The formula for type 1 diabetes was as follows: HbA$_{1c}$ (%) = 0.028 × MPG (mg/dL) + 2.77 (R^2 = 0.77). The formula for type 2 diabetes was as follows: HbA$_{1c}$ (%) = 0.028 × MPG (mg/dL) + 2.62 (R^2 = 0.82).

The clinical and glycemic characteristics are shown in Table 1. Mean HbA$_{1c}$ (SD) for type 1 diabetic patients was 7.3% (1.1) and for type 2 diabetic patients was 6.8% (1.1).

All GV measures had significant influence on the MPG-HbA$_{1c}$ relationship for the total population. The variability index SD showed the strongest influence on the MPG-HbA$_{1c}$ relationship. None of the GV measures showed confounding for all diabetic patients pooled or for the type 1 or type 2 diabetic patients separately (Table 2).

In the type 1 diabetic patients, the effect modification of SD and CONGA$_4$ was significant (P < 0.01 and P = 0.02), and for the MAGE it was just not significant (P = 0.06) (Table 2). The MPG/HbA$_{1c}$ linear regression formula with confounding for type 2 diabetes was as follows: HbA$_{1c}$ (%) = 2.64 + 2.63 × MPG/100 + 0.58 × SD/100. The MPG-HbA$_{1c}$ linear regression formula with effect modification for type 1 diabetes was as follows: HbA$_{1c}$ (%) = 3.91 + 1.79 × MPG/100 − 1.37 × SD/100 + 1.25 × MPG/100 × SD/100. The impact of effect modification of low GV (SD = 30 mg/dL) versus high GV (SD = 100 mg/dL) for an MPG level of 160 mg/dL in type 1 diabetes on the HbA$_{1c}$ level was 6.96 vs. 7.41%, respectively, as shown in Table 3. At an MPG level of 220 mg/dL (HbA$_{1c}$ following the regression formula of 8.89%), a decline in the SD parameter from 100 to 30 mg/dL reduced HbA$_{1c}$ from 9.23 to 8.26%.

Table 1—Baseline clinical and glycemic characteristics

	All	Type 1 diabetes	Type 2 diabetes
n	427	268	159
Age (years)	47.6 ± 13.6	44.1 ± 12.9	56.6 ± 9.4
Sex (% female)	54	52	51
Ethnicity (% non-Hispanic whites)	83	93	73
Current smokers	11	12	9
Insulin treatment	76	100	38
Glycemic measures			
HbA_{1c} (%)	6.8 ± 1.3	7.3 ± 1.1	6.8 ± 1.1
MPG (mg/dL)	149.4 ± 39.6	162 ± 36	149.4 ± 36
Measures of GV			
CGM SD (mg/dL)	48.6 ± 25.2	64.8 ± 16.2	39.6 ± 16.2
MAGE (mg/dL)	86.4 ± 43.2	115.2 ± 32.4	68.4 ± 27
$CONGA_4$ (mg/dL)	66.6 ± 28.8	88.2 ± 23.4	52.2 ± 21.6
SD (mg/dL)			
≤30	61 (14.3)	9 (3.4)	52 (32.7)
<30–60	173 (40.5)	84 (31.3)	89 (56)
<60–90	173 (40.5)	155 (57.8)	18 (11.3)
>90	20 (4.7)	20 (7.5)	0 (0)

Data are means ± SD, %, or n (%).

For all patients pooled, there was no effect modification of the respective GV measures on the MPG-HbA_{1c} relationship. For type 2 diabetic patients, the impact of effect modification from the respective GV measures was far from significant (Table 2). The number of patients with a predefined SD is shown in Table 1 for all patients pooled and for the type 1 and type 2 diabetic patients separately.

CONCLUSIONS—This study demonstrated a significant effect of GV, as reflected by SD, on the MPG-HbA_{1c} relationship. High GV (SD) is associated with higher HbA_{1c} levels for a given MPG, and this effect was more pronounced at higher HbA_{1c} and MPG values. However, the magnitude of this effect of GV was small and only demonstrable in type 1 diabetic patients. Possibly, the type 2 diabetic patient group was too small (n = 159) and the variability in this group too low to find this interaction.

The ADAG study showed a tight correlation between HbA_{1c} and MPG, allowing the translation of HbA_{1c} into estimated average glucose (14,19). It has been suggested that GV could affect the MPG-HbA_{1c} relationship, but this has not previously been demonstrated (20–22). To our knowledge, the current study is the largest study reporting an influence of GV—as expressed by SD, MAGE, and $CONGA_4$ calculated from CGM—on the MPG-HbA_{1c} relationship. The discrepancies in the MPG-HbA_{1c} relationship are less likely caused by technical errors because this study included accurate and centralized measurements of HbA_{1c} values and intensively measured plasma glucose concentrations (~2,700 values) in a large and diverse population. Also, individuals with conditions or treatment that might result in major changes in glycemia or interference with the HbA_{1c} assay, or the MPG-HbA_{1c} relationship, were excluded. These precautions allowed us to search for factors other than MPG that may contribute to HbA_{1c}.

In general, GV is higher in patients with poor glycemic control and in type 1 diabetic patients compared with type 2 diabetic patients, which can be attributed to insulin therapy and higher insulin sensitivity. High GV may affect glycation because of periodic exposure of the erythrocyte to high glucose levels and therefore to faster irreversible glycation.

Other factors like hyperglycemia-induced oxidative stress may affect the glycation process. In recent literature, it has been speculated that oxygen free radicals per se or with an associated decrease in antioxidants may modulate the formation of early glycated protein (10,11).

Brownlee (12) demonstrated that hyperglycemia stimulates oxidative stress. High GV and especially postprandial glucose excursions were also previously

Table 2—The P values of the influence of the respective GV measures themselves, as well as effect modification and the Δ of confounding, calculated from the respective slopes (B and B′) from the regression equations, on the HbA_{1c}-MPG relationship for all patients pooled and for type 1 and type 2 diabetic patients separately

	Influence of the GV measure (P)	Slope of MPG (B) in the main regression formula	Slope of MPG (B′) in the regression formula with the GV measure	ΔConfounding (%)*	Effect modification (P)
SD					
All	<0.01	2.818	2.624	6.9	0.06
Type 1 diabetic	0.01	2.781	2.631	5.4	<0.01
Type 2 diabetic	0.06	2.782	2.637	5.2	0.74
MAGE					
All	<0.01	2.818	2.700	4.2	0.37
Type 2 diabetic	0.19	2.781	2.721	2.2	0.06
Type 2 diabetic	0.19	2.782	2.698	3.0	0.19
$CONGA_4$					
All	<0.01	2.818	2.667	5.4	0.15
Type 1 diabetic	0.06	2.781	2.687	3.4	0.02
Type 2 diabetic	0.07	2.782	2.661	4.3	0.46

*ΔConfounding in % = 100 × absolute (B′ − B)/B.

Table 3—*Quantification or impact of confounding or effect modification for type 1 diabetic patients of a low vs. a high SD for a given MPG on the HbA₁c level next to the HbA₁c values calculated with the regression formula for type 1 diabetes*

MPG [mg/dL (mmol/L)]	Regression formula	Confounding		Effect modification	
		SD 30 mg/dL	SD 100 mg/dL	SD 30 mg/dL	SD 100 mg/dL
140 (7.8)	6.67	6.50	6.90	6.53	6.80
160 (8.9)	7.22	7.02	7.43	6.96	7.41
180 (10)	7.78	7.55	7.95	7.39	8.02
200 (11.1)	8.34	8.08	8.48	7.83	8.62
220 (12.2)	8.89	8.60	9.01	8.26	9.23
240 (13.3)	9.45	9.13	9.53	8.69	9.84

The header "HbA₁c (%)" spans the results columns.

associated with oxidative stress in type 2 diabetes (13). The activation of oxidative stress, estimated from urinary excretion rates of isoprostanes, was highly correlated with MAGE calculated from CGM (13). However, Wentholt et al. (23) could not replicate these results in type 1 diabetes. Recently, Ceriello et al. (15) demonstrated that high intraday GV was more damaging to endothelial function than stable hyperglycemia and that oxidative stress plays a key role. Whether oxidative stress influences glycation needs to be determined.

On the other hand, it has been demonstrated that erythrocyte survival is shorter at chronic high glucose concentrations levels, which might falsely lower HbA₁c levels. Peterson et al. (24) showed that the life span of 51Cr-labeled erythrocytes increased in all seven subjects when their poorly controlled diabetes was adequately treated. Virtue et al. (25) concluded that there is a hyperglycemia-related decrease in erythrocyte survival as measured by carbon monoxide in the expired air, which results in an exponential underestimation of the severity of hyperglycemia at higher HbA₁c levels. Similarly, hyperglycemia-related osmotic stress may influence erythrocyte permeability and could cause damage to the erythrocyte and shorten its life span. These findings could lead to underestimation of HbA₁c at higher MPG levels, concealing a glycemic control worse than indicated by HbA₁c measurements. However, we found that type 1 diabetic patients with high GV display higher HbA₁c levels than suspected based on MPG. This effect was more pronounced at higher HbA₁c levels, indicating that focus on reducing GV, especially in patients with poor glycemic control, could help reduce HbA₁c levels.

Limitations of our study are that CGM has a limited range of reliable measurements between 2.2 mmol/L and 22.2 mmol/L. Therefore, theoretically, CGM performance could be less precise in patients with high glycemic variability. Furthermore, CGM has a lag time in glucose values compared with the venous measured values (the physiological gap), and this can result in underestimation of the influence of GV on the glycation of HbA₁c, and no measures of erythrocyte survival, oxidative stress, or clinical follow-up are available in this population.

In conclusion, at higher levels of GV the relationship between HbA₁c and MPG in patients with type 1 diabetes is altered, leading to a higher HbA₁c level for a given MPG. However, the impact (near the HbA₁c treatment target of 7%) is only modest.

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