

Relationship between glycated haemoglobin levels and mean glucose levels over time

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Abstract

Aims/hypothesis HbA_{1c}, expressed as the percentage of adult haemoglobin that is glycated, is the most widely used measure of chronic glycaemia. Achieving near-normal HbA_{1c} levels has been shown to reduce long-term complications and the HbA_{1c} assay is recommended to determine whether treatment is adequate and to guide adjustments. However, daily adjustments of therapy are guided by capillary glucose levels (mmol/l). We determined the relationship between an accurate measure of mean glucose levels over time and the HbA_{1c} level, and whether HbA_{1c} can be expressed in the same units as self-monitoring results.

Methods Twenty-two participants with diabetes and three non-diabetic participants were included in this longitudinal observational study. Mean glucose levels were measured by continuous glucose monitoring (CGM), which measures interstitial glucose levels every 5 min, for 12 weeks. Capillary measurements were obtained four times per day to confirm the accuracy of CGM. HbA_{1c} was measured at baseline and every 4 weeks.

Results The HbA_{1c} results at weeks 8 and 12 correlated strongly ($r=0.90$) with the CGM results during the preceding 8 and 12 weeks. A curvilinear (exponential) relationship and a linear regression captured the relationship with similarly high correlations, which allowed transformation of HbA_{1c} values to a calculated mean glucose level.

Conclusions and interpretation HbA_{1c} correlates closely with a complete measure of average glycaemia over the preceding 8–12 weeks. The translation of HbA_{1c} to an

average glucose level for reporting and management purposes is feasible.

Keywords Diabetes · Diabetes management · Chronic glycaemia · Glycated haemoglobin

Abbreviations

CGM continuous glucose monitoring
CGMS Continuous Glucose Monitoring System
MBG mean blood glucose concentration
NGSP National Glycohemoglobin Standardization Program

Introduction

The HbA_{1c} assay has become the most commonly used measure of chronic glycaemia in epidemiological studies, clinical trials and the management of diabetes since its introduction more than 25 years ago [1–3]. The concentration of HbA_{1c}, formed through the non-enzymatic attachment of glucose to haemoglobin, is commonly considered to reflect the integrated mean glucose level over the previous 8–12 weeks, the time period being dictated by the 120 day lifespan of the erythrocyte. The relationship between mean blood glucose and HbA_{1c} has been suggested by old studies that used a variety of measures of outpatient and inpatient plasma and capillary glucose concentrations, all of which suffered from relatively infrequent sampling of glucose levels, infrequent sampling of HbA_{1c}, or both [4–8]. Arguably, the two most convincing studies measured four to six fingerstick blood glucose levels per day for 6–8 weeks and compared the calculated mean blood glucose levels with the HbA_{1c} levels measured at the end of the study period [9, 10]. The clinical role of the assay was subsequently established by demonstrating that it provided information regarding mean blood glucose that could not be provided by any other available measures [10]. The pre-eminent role of

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the assay was cemented by the Diabetes Control and Complications Trial (DCCT), and others, which used the assay to measure chronic glycaemia and demonstrated its central role in the pathogenesis of long-term complications [11–13]. The recommended goals for metabolic control of diabetes are based on the HbA_{1c} assay [2, 14, 15], the vast majority of methods now being standardised to the DCCT assay through an international programme [16].

Despite the popularity and central role of the HbA_{1c} assay, its true relationship to mean blood glucose remains poorly understood. HbA_{1c} appears to reach a new steady state 3–4 months after glucose changes [17]. All of the previous studies that suggested a relationship between HbA_{1c} level and mean blood glucose [4–10] used relatively infrequent monitoring during the daytime, which is prone to sampling error, and did not capture glucose levels overnight or true 24 h glycaemia. In addition, the limited time period of the previous studies and limited number of HbA_{1c} assays performed precluded an analysis of the time period of mean glycaemia that the HbA_{1c} reflects best.

We used continuous glucose monitoring (CGM), which measures interstitial glucose levels every 5 min, for 3 months in patients with diabetes with relatively stable glycaemia, and in non-diabetic individuals, to determine true mean glycaemia. HbA_{1c} assays were measured monthly and best-fit correlations were determined over time.

Methods

Participants Patients with type 1 ($n=15$) or type 2 ($n=7$) diabetes mellitus were recruited from the Massachusetts General Hospital Diabetes Center. Three non-diabetic volunteers were also recruited. The diabetic participants were required to have relatively stable glycaemic control, manifest by at least two HbA_{1c} assay results in the previous 6 months that were no more than 1% different. We tried to recruit approximately similar numbers of diabetic participants with HbA_{1c} <7, 7–8.5 and >8.5%. The non-diabetic participants had no history of diabetes and had HbA_{1c} levels in the non-diabetic range (<6.1%) in the assays used for the study. Exclusion criteria included any condition that would probably cause a change in glucose control during the 3 month period of study or that would change erythrocyte turnover. Potential participants with known haemoglobinopathies were excluded to avoid any possible interference with the HbA_{1c} assays [18]. All participants signed an informed consent form approved by the Human Research Committee.

Measurements CGM was performed with the Continuous Glucose Monitoring System (CGMS Medtronic Minimed, Northridge, CA, USA), which measures interstitial glucose

levels every 5 min through a catheter inserted subcutaneously. Participants changed the sensor units every 3 days, as instructed by the manufacturer, and returned the monitors to the Diabetes Center every 2 weeks so that they could be downloaded. Diabetes therapy was not adjusted on the basis of the CGM data, unless otherwise unrecognised periods of hypoglycaemia, such as periods of nocturnal glucose levels <3.33 mmol/l, were noted. When this occurred, the patient's physician was notified so that appropriate treatment changes could be made. In addition to the CGM, participants were requested to perform self-monitoring of capillary glucose (OneTouch Ultra; Lifescan, Milpitas, CA, USA) at least four times per day, usually before meals and at bedtime. These measurements were used to calibrate the CGMS and adjust therapy, and provided an independent measure of glycaemia. Capillary glucose levels are calibrated by Lifescan to provide values equivalent to plasma glucose levels. Glucose levels were also measured with a laboratory-based hexokinase assay (Hitachi Model 917 autoanalyser; Hitachi, Tokyo, Japan) at each 2 week visit to the Diabetes Center, contemporaneously with a self-glucose monitoring test. The capillary glucose values correlated closely with the simultaneously measured laboratory values ($n=146$ pairs, capillary value = $0.8876 \times \text{laboratory value} + 21.4$, $R^2 = 0.9629$, $p < 0.001$), validating the accuracy of the self-monitoring. The mean absolute difference between the self-monitored and laboratory results was <0.11 mmol/l.

Two different assays were used to measure HbA_{1c}: an HPLC method that is the primary reference method for the National Glycohemoglobin Standardization Program (NGSP) [15] and a boronate affinity assay (Primus PDQ, Primus Diagnostics, Kansas City, MO, USA) that measures HbA_{1c} independently of any haemoglobin abnormalities. Because of technical issues (modestly elevated fetal haemoglobin levels in five participants and missing samples at several time points for two participants), several participants' samples could not be assayed accurately with the NGSP reference assay [18]. Therefore, the primary results presented here are from the boronate affinity assay. Both HbA_{1c} assays have CVs <2% at low (5%) and high (10%) HbA_{1c} values in our laboratory. Long-term calibration standards are used to prevent assay drift in the NGSP HPLC method and the boronate affinity assay is calibrated against it. The correlation of the boronate affinity assay with the primary HPLC assay is $r=0.99$ ($p < 0.001$).

Statistical analyses The demographic data are expressed as mean $\pm$ SD. Correlations between mean glucose levels derived from the CGM results and HbA_{1c} levels were analysed using linear regression, exponential and quadratic equations, with Spearman correlations. The best fits, judged by the r values closest to 1.0, were determined. Analyses were performed using Stata statistical software (Stata

Statistical Software Release 8.0, 2003; Stata Corporation, College Station, TX, USA).

Results

Twenty-nine eligible volunteers were recruited to the study. Three volunteers withdrew within the first few weeks because of their inability to carry out various elements of the study. A fourth volunteer was hospitalised for medical reasons unrelated to the study and could not complete the study in a timely fashion. Baseline characteristics of the 25 participants who completed the 12 week study are shown in Table 1.

The participants had HbA_{1c} measured four times, at baseline and monthly for 3 months. HbA_{1c} levels during the study changed minimally from baseline ($7.39 \pm 1.4\%$) to study end ($7.16 \pm 1.3\%$), with the greatest absolute difference $<1\%$. CGM was performed on average for 81.8 ± 4 days of the 12-week period for the diabetic participants, representing 97% of the possible 84 day period and approximately 24,000 glucose measurements per participant. Self-monitoring by capillary fingerstick was performed an average of 3.73 ± 1.93 times per day by the diabetic participants and 2.68 ± 1.08 times per day by the non-diabetic participants. Almost 6000 paired (simultaneous capillary and interstitial CGMS tests) assays were performed.

The correlations between mean CGM glucose levels and HbA_{1c} levels at the different time points during the study are shown in Table 2. The strongest correlations (Spearman $r=0.89-90$, $p<0.001$) were between the mean CGM glucose levels measured during the entire 12-week period and the HbA_{1c} level at weeks 8 or 12.

The linear and exponential models provided similarly close correlations between the CGM during the 12 weeks of the study and the HbA_{1c} value at 12 weeks: linear model, mean CGM concentration (mean CGM)=HbA_{1c} $\times 1.75-3.81$ ($r=0.89$, $p<0.001$) (Fig. 1a); exponential model, mean CGM= $1.28\text{HbA}_{1c}+0.000136\exp(\text{HbA}_{1c})-0.92$ ($r=0.89$, p

Table 1 Baseline characteristics of study participants

	Type 1 diabetes	Type 2 diabetes	Non-diabetic	All
<i>n</i>	15	7	3	25
Age (years)	49 ± 10.1	64 ± 9.8	43 ± 18.8	52 ± 13.2
Sex (% women)	53	57	67	56
Diabetes duration (years)	28.3 ± 11.6	21.4 ± 8.7	N/A	26 ± 11.0
Race (% white)	87	86	100	93
HbA _{1c} (%)	7.6 ± 1.0	8.1 ± 1.4	4.9 ± 0.3	7.4 ± 1.4

Data are mean $\pm$ SD

Table 2 Correlation of HbA_{1c} with mean continuous glucose level

	HbA _{1c} level at		
	Week 4	Week 8	Week 12
Mean CGM at:			
Weeks 1–4	0.83	0.81	0.79
Weeks 5–8	0.81	0.88	0.87
Weeks 9–12	0.81	0.88	0.90
Weeks 1–8	0.86	0.89	0.87
Weeks 1–12	0.86	0.90	0.89

Data are Spearman correlation coefficients; all $p<0.001$

<0.001) (Fig. 1b). The correlations of mean CGM levels with the HbA_{1c} values assayed with the NGSP reference HPLC method had an r value for the linear regression of 0.90 ($p<0.001$).

The HbA_{1c} values were transformed into their equivalent mean CGM values using the exponential and linear regression equations (Table 3). Similar transformations with the HPLC assay results provided similar mean glucose values (not shown).

Discussion

The HbA_{1c} assay is widely accepted as the best means of retrospectively capturing mean glycaemia. It is the basis of treatment guidelines and is used universally to adjust therapy [2, 14, 15]. In order for patients to achieve HbA_{1c} goals, they adjust their day-to-day therapies based on fingerstick capillary glucose levels, measured with meters that are adjusted to provide values comparable to venous plasma levels [3]. However, the relationship between HbA_{1c} values and mean glucose levels has never been carefully explored, owing in great part to the absence of means to measure glucose levels frequently enough to reveal a complete description of mean glucose levels over time. Previous studies that have been performed have used relatively infrequent glucose testing and have not been able to measure mean blood glucose levels with confidence because of potential sampling errors [4–10]. For example, the DCCT data set included a seven-point glucose profile performed every 3 months [8]. The intra-individual variations in mean glucose profiles and HbA_{1c} were 29.9 and 9.7% respectively, suggesting the relatively high degree of variability of glucose profiles collected intermittently, and reinforcing the need for frequent measurements to capture mean glycaemia.

We took advantage of the development of CGM devices, which measure interstitial glucose levels every 3–5 min. By using these devices over a 3 month period, we provided a more complete measure, including approximately 24,000 glucose tests per participant, of the average glucose levels

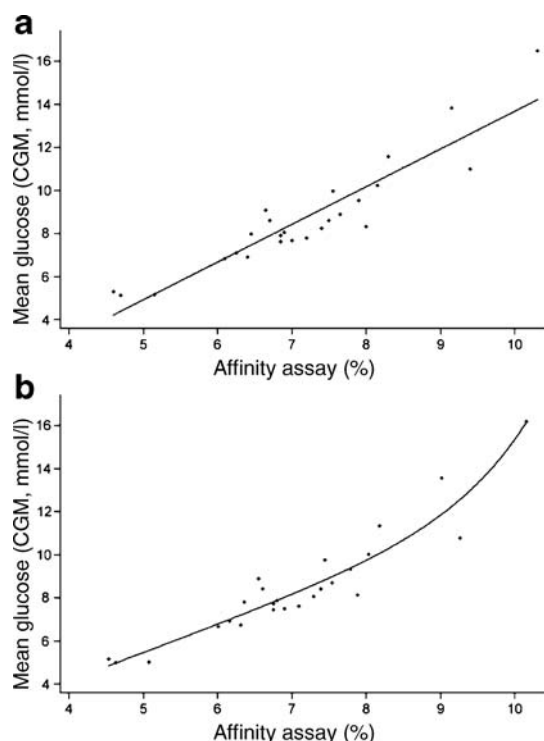


Fig. 1 Relationship between HbA_{1c} at month 3 and mean glucose level calculated from CGM during 12 previous weeks according to (a) linear regression mean CGM = $\text{HbA}_{1c} \times 1.75 - 3.81$ ($r = 0.89$, $p < 0.001$), and (b) the exponential mean CGM = $1.28\text{HbA}_{1c} + 0.000136\exp(\text{HbA}_{1c}) - 0.92$ ($r = 0.89$, $p < 0.001$). Continuous line, fitted values; diamonds, observed values

in 25 diabetic and non-diabetic individuals and determined their relationship with HbA_{1c}. Although some studies have suggested less than acceptable correlations between CGMS interstitial glucose results and venous plasma or capillary glucose levels, most recent studies, including the present study, have noted generally strong correlations [19, 20]. The results showed that HbA_{1c} is tightly correlated with mean interstitial glucose levels, with exponential and linear regression equations providing similarly tight fits for the data. Previous studies with less detailed measures of chronic glucose values, including one using intermittent CGM, have also suggested a curvilinear relationship with HbA_{1c} [9, 21]. The limited number of HbA_{1c} assay results in the higher range limits our ability to choose between the linear and exponential models. Although the choice of research participants with stable HbA_{1c} levels and the decision not to adjust therapy during the study limited our ability to examine the dynamics of glucose change and HbA_{1c} levels, the strongest correlations between HbA_{1c} and mean glucose levels appeared to be over the preceding 12 weeks, consistent with prior studies [17].

Of note, although there was less than a perfect correlation between the measured mean glucose levels and the HbA_{1c} levels, the relatively high r values, especially given the limited sample size and range of values, suggests

that there are probably no extraneous factors that affect the relationship between MBG and HbA_{1c} in a substantive way. Some studies have suggested that variable red cell turnover, which may be affected by hyperglycaemia, may alter the relationship between mean blood glucose and HbA_{1c} values [22]. Other studies have proposed that glycation rates may differ among individuals at the same mean glucose levels [23, 24]. However, the studies that have supported differential rates of glycation (high vs low glycaters) have generally been performed in non-diabetic individuals with very limited glucose testing. A study by Hempe and colleagues in 128 children with type 1 diabetes suggested that differential glycation occurred since mean blood glucose levels during a 1 month period, assessed with approximately 80 self-monitored values, did not correlate tightly with the HbA_{1c} at the end of the month ($r = 0.71$) [25]. However, the present study and others [17] strongly suggest that a 1-month period is inadequate for HbA_{1c} to equilibrate and that if more complete glucose monitoring is performed (8000 assays in 1 month in our study compared with 80 in the Hempe study) the correlations become substantially tighter. The absence of any major outliers in the present study suggest that if differential glycation exists it is relatively rare, or does not affect the relationship between blood glucose values and HbA_{1c} levels enough to disturb a predictable relationship between the two. However, considering the small sample size in the present study and the relatively homogeneous study population, most of the participants being white and with type 1 diabetes, larger studies using comparable assessment of chronic glycaemia will need to be performed to determine conclusively whether differential glycation exists.

Table 3 Mean glucose equivalent values for HbA_{1c}

HbA _{1c} (%)	Predicted CGMS (mmol/l)	
	Linear ^a	Exponential ^b
5	4.94	4.67
5.5	5.78	5.22
6	6.67	5.83
6.5	7.56	6.44
7	8.44	7.06
7.5	9.28	7.67
8	10.2	8.39
8.5	11.1	9.22
9	11.9	10.2
9.5	12.8	11.5
10	13.7	13.2
10.5	14.6	15.7
11	15.4	19.5

^a Based on linear regression mean CGM = $\text{HbA}_{1c} \times 1.75 - 3.81$ ($r = 0.89$, $p < 0.001$)

^b Based on exponential mean CGM = $1.28\text{HbA}_{1c} + 0.000136\exp(\text{HbA}_{1c}) - 0.92$ ($r = 0.89$, $p < 0.001$)

The relatively small number of participants precludes any expansive conclusions regarding the relationship between HbA_{1c} and mean glucose levels in the larger diabetic population. Similarly, the limited range of HbA_{1c} levels in the present study (the highest HbA_{1c} level was <11%) does not allow extrapolation of our results to patients with HbA_{1c} levels that are very high. However, we are confident of our findings in the range of glycaemia studied, and have therefore restricted the conversion table (Table 3) to the values included in the study. While inclusion of patients with higher HbA_{1c} levels will be required to determine the conversion equations covering a wider range of glycaemia, and whether an exponential model fits better than a linear one, the range of HbA_{1c} levels in the present study applies to the vast majority of diabetic patients. Of note, similar correlations between average chronic glycaemia and HbA_{1c} were obtained with all of the HbA_{1c} assays performed; thus, the relationship is not limited to only one HbA_{1c} assay.

The mathematical relationship established between HbA_{1c} and mean blood glucose in this study should allow the expression of HbA_{1c} as an equivalent mean glucose level. This is likely to be beneficial to patients and care providers alike as the assay of chronic glucose control would be in the same units as the patients' self-monitoring. However, before this transformation can take place, the mathematical relationship between HbA_{1c} and mean glucose levels should be confirmed in a larger study with a more diverse population to ensure that the relationship applies for all patients with diabetes. An international study that is examining the relationship between mean glucose levels and HbA_{1c} levels in a large ($n=700$), multinational study population including non-diabetic participants and type 1 and type 2 diabetic participants is ongoing. Like the present study, the international study is employing CGMS and frequent self-monitored capillary profiles to capture average glucose levels as completely as possible.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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