The relationship of A1c to glucose concentrations in children with type 1 diabetes: Assessments by high frequency glucose determinations by sensors.

The Diabetes Research in Children Network (DirecNet) Study Group

Running title: A1c and Mean Glucose

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ABSTRACT

Objective: Despite its standing as the most validated and widely used measure for average glycemic control over time, the relationship between hemoglobin A1c (A1c) and glucose concentrations is not completely understood. The purpose of this DirecNet study was to utilize continuous glucose monitoring data to examine the relationship between A1c and glucose in type 1 diabetes.

Research Design and Methods: 48 youth enrolled in studies of Navigator continuous glucose monitor were encouraged to wear the Navigator sensor at home continuously. A1c was measured at baseline, 3 months, and 6 months. Sensor glucose data were directly transmitted via the internet, assuring that essentially all glucose values were analyzed.

Results: Subjects had a median of 112 hr/wk of Navigator data in the first 3 months and 115 in the second 3 months. The slope of mean glucose over the previous 3 months vs. A1c was only 18 mg/dL per 1.0% A1c. Individually, there was substantial variation in the relationship between mean glucose and A1c. A1c was not associated with glucose lability after controlling for mean glucose. Measures of an individual’s rate of glycation were moderately correlated at the 3 and 6-month visits.

Conclusions: As the chemistry of glycation would predict, we found no evidence to contradict the simple hypothesis that A1c directly reflects the mean glucose over time. There is, however, substantial variability in individual mean glucose concentrations for a given A1c. Transforming reliable A1c values into calculated mean glucose values would, when applied to an individual, introduce substantial error.
Despite its standing as the most validated and widely used measure for average glycemic control over time, the relationship between hemoglobin A1c (A1c) and blood glucose concentrations is not completely understood. The chemistry of this first order, non-enzymatic glycation reaction should reflect the integral of glucose concentration over time, adjusted for the survival of red blood cells, but this simple relationship has been questioned by many clinicians and investigators, as discussed by Cohen et al (1). Some clinicians hold that higher glucose concentrations increase the A1c more than lower glucose levels reduce it. Some studies have indicated that post-prandial glucose values have a greater impact on A1c than do pre-prandial glucose concentrations (2), while others suggest that the opposite is true (3). Even assuming that A1c linearly reflects a time weighted average of blood glucose concentrations, the exact ratio between average blood glucose and A1c remains uncertain, with published data indicating that a one percentage point rise in A1c is the equivalent to anywhere from a 19-36 mg/dL rise in average glucose concentrations (4, 5).

Many factors contribute to different estimates of the slope of the relationship between average plasma glucose and A1c. One major reason for this uncertainty stems from limitations in determining the average glucose level using traditional conventional home glucose monitoring systems. Typically, glucose is measured infrequently, and patients often over-sample when they are symptomatic or when a measurement is outside their target range and under-sample at night.

Another unresolved and controversial issue is whether individuals glycate hemoglobin proteins at different rates. Among 223 adults without diabetes, differences in glucose intolerance explained only one third of the variance found in glycated hemoglobin levels (6). Comparing non-diabetic monozygotic and dizygotic twins, Cohen et al estimated that 69% of the variance in the glycation gap was heritable (7). This question has taken on added importance due to the recent suggestion that higher glycation rates may predict increased risk for long-term complications independently of glycemic control (8), though this is controversial (9).

The Diabetes Research in Children Network (DirecNet) has carried out two, non-randomized studies to evaluate the efficacy of the FreeStyle Navigator™ Continuous Glucose Monitoring System (“Navigator”, Abbott Diabetes Care, Alameda, CA) in children and adolescents with type 1 diabetes (T1D) treated with insulin pump and glargine-based multiple daily injection regimens. These studies involved subjects who varied widely with respect to A1c levels. The wealth of continuous glucose monitoring data that was generated by these 6-month studies provided us with a unique opportunity to examine the relationship between A1c and glucose concentrations in youth with diabetes.

**METHODS**

The procedures for the Navigator studies have been described in detail elsewhere (10) and are briefly summarized here.

Fifty-seven subjects with T1D, age 4-<18 y, A1c 5.8-10.3%, were enrolled into non-randomized pilot studies of Navigator use in pump (n=30) and glargine-based multiple daily injection (MDI, n=27) regimens. Ninety-three percent were Non-Hispanic white. This system combines a glucose sensor with a built in FreeStyle glucose meter, which was used to calibrate the Navigator four times, at approximately 10, 12, 20 and 48 hours after insertion. Subjects were encouraged to wear the Navigator sensor at home as often as possible. A1c was measured at baseline, 3 months, and 6 months with the DCA 2000® + Analyzer (Bayer, Inc.,
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Tarrytown, NY). We have previously shown that A1c levels measured by the DCA2000 are highly correlated \( r = 0.94 \) with reference A1c levels measured in the DCCT/EDIC Laboratory at the University of Minnesota, albeit with slightly higher values (mean difference +0.2% vs. DCCT/EDIC Laboratory with 95% confidence interval of +0.14 to 0.23%, \( p < 0.001 \)) (11). The median relative absolute difference was 0.03.

Sensor glucose data were directly transmitted from the subject’s home to the DirecNet Coordinating Center, assuring that essentially all glucose values were available for analysis.

**Statistical Methods.** Analysis of mean glucose measured by the Navigator sensor vs. A1c measured by the DCA2000 combined data from the 3 and 6-month visits. A visit was included in analysis if the subject had at least 24 hours of Navigator data for at least 6 (not necessarily consecutive) of the 13 prior weeks. One outlier at the 3-month visit with a mean glucose of 233 mg/dL and an A1c of 8.3% was excluded from analysis (results were similar whether or not this data point was included). Forty seven subjects (28 pump users and 19 using MDI-glargine) met this criterion for the 3-month visit, and 28 subjects (17 pump users and 11 using MDI-glargine) met this criterion for the 6-month visit. The combined analysis included 75 visits from 48 subjects. All statistical analyses were conducted using SAS version 9.1 software (SAS Institute Inc., Cary, NC).

The mean glucose from the sensor data was calculated giving equal weight to each of the 24 hours of the day. Repeated measures least squares regression was performed using mean glucose as the dependent variable and A1c as the independent variable combining data from the 3 and 6-month visits. There were no significant interactions with visit time (3 vs. 6 months) or insulin modality (pump vs. MDI).

Two measures of glycation were calculated from the sensor data: first a simple ratio of mean glucose to the A1c and second the hemoglobin glycation index (HGI) defined as the residual from a regression model with A1c as the dependent variable and mean glucose as the independent variable (8). These measures are intended to quantify an individual’s tendency to glycate, and may be helpful in predicting an individual’s future risk for complications. Pearson correlation was used to assess the consistency of these measures between 3 and 6 months.

Additional regression models were run with A1c as the dependent variable to see if any measure of glucose lability was predictive after adjusting for mean glucose. Lability measures included mean amplitude of glycemic excursions (MAGE) (12), absolute rate of change (13) and percentage of glucose values >250 mg/dL and >300 mg/dL. Separate models were run testing these lability measures one at a time adjusting for mean glucose.

**RESULTS**

The 47 subjects (28 pump users and 19 using MDI-glargine) whose 3-month visits were included in the analysis had a median (25th - 75th percentiles) 112 (83 -130) hours of Navigator data per week. The 28 subjects (17 pump users and 11 using MDI-glargine) whose 6-month visits were included in the analysis had a median (25th - 75th percentiles) 115 (88 - 131) hours of Navigator data per week.

The slope (± 95% CI) of mean glucose over the previous 3 months vs. A1c was 18 ± 4 mg/dL per 1.0% A1c. Moreover, for a given A1c, mean glucose concentrations varied considerably (Figure 1). For example, for an A1c of 7%, mean glucose concentrations ranged from 138 to 189 mg/dL. This relation was similar for subjects using a pump or MDI-glargine and at the 3 and 6-month visits (Figure 1).
Measures of an individual’s rate of glycation were moderately correlated at the 3 and 6-month visits (Figure 2). The Pearson correlation was 0.70 (95% confidence interval: 0.42 to 0.85) for the ratio of mean glucose (over the previous 3 months) to A1c and 0.63 (0.33 to 0.81) for the HGI. It is noteworthy that the slope of the relationship between the change in mean sensor glucose and change in A1c between 3 and 6 months was 12 ± 9 mg/dL (Figure 3).

There was no evidence to suggest that the rate of glycation changed non-linearly with glucose concentration. After controlling for the mean glucose over the prior 3 months, A1c was not significantly associated with measures of glucose lability (MAGE: p=0.33, absolute rate of change: p=0.76, percentage of glucose sensor readings >250mg/dL: p=0.18, percentage of glucose sensor readings >300 mg/dL: p=0.39).

DISCUSSION

By the design of our data capture system, we were able to collect and analyze essentially all sensor glucose values obtained by our subjects. Just as chemistry of glycation would predict, we found no evidence to contradict the simple hypothesis that A1c directly reflects the integral of glucose level over time, often referred to as the area under the curve (AUC). Thus high glucose values, such as those typically seen post-prandially, do not disproportionately contribute to A1c. Moreover, glucose variability does not appear to impact the A1c. These results are similar to those of Salardi et al. using the retrospective CGMS device from MiniMed (14).

The slope of the regression equation relating A1c to mean glucose in this study (18 mg/dL for each one percentage change in A1c) is lower than found by others. Rohlfing et al. using data from the DCCT, found a slope of 36 mg/dL (4). In a recent small study, Nathan et al. found a tighter association between mean glucose and A1c and a slope of 32 mg/dL. Their adult subjects included patients with both type 1 and type 2 diabetes as well as subjects without diabetes (15). The slope observed in this study is consistent with that which we have previously reported in 200 youths with T1D involved in the DirecNet randomized clinical trial of the GlucoWatch G2 Biographer that was based on either 3 days of 8-point meter testing (i.e., 1% A1c change for every 23 mg/dL change in mean plasma glucose) or 3 days of continuous glucose monitoring using the Medtronic MiniMed CGMS (i.e., 1% A1c change for every 19 mg/dL change in mean plasma glucose) (5).

Of note, the subjects in the DirecNet studies are children and adolescents who may have higher glycemic variability than adults. Our findings may not therefore be directly applicable to adult patients. The difference in slope, however, has implications for the care of patients with diabetes. A smaller improvement in average glucose concentration translates, on average, to a larger improvement in A1c. Moreover, in this study there is substantial variability in individual mean glucose concentrations for a given A1c. For any given A1c level, mean sensor glucose levels differed by up to 50 mg/dL or more (Figure 1), making the conversion of A1c levels into mean glucose equivalents as suggested by a recent ADA consensus statement (16) tenuous at best.

An additional aim of this study was to use our continuous glucose monitoring data to explore whether and to what extent rates of glycation or turn over of glycosylated hemoglobin vary among individual patients. It is also particularly important that both measures of glycatability were reasonably constant over the six months of our study, consistent with the hypothesis that individual subjects with diabetes glycate proteins at differing rates and that this tendency to be a fast or slow glycator persists over time within
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Individual differences in glycatability explained <50% of the differences in A1c levels between patients and a number of other factors, such as differences in red blood cell turnover or the inter-assay coefficient of variation of A1c measurements, undoubtedly play a role. Herman et al, using data from the Diabetes Prevention Program, have recently reported that A1c, adjusted for glycemic indices, varies by race and ethnicity (17).

Within our DirecNet trials, we have demonstrated the A1c measurements, a recognized valid measure of diabetes control, can be quite reliable. We found that A1c obtained with the Bayer’s DCA 2000 matched reference method closely (11). Some have suggested that transforming these reliable A1c values into calculated mean glucose values would improve diabetes management. While A1c clearly reflects mean glucose, our data demonstrate this approach, when applied to an individual, could introduce substantial error. Moreover, A1c may be such a strong surrogate marker for the risk of diabetic complications because it reflects both the average plasma glucose level and the propensity of the patient to glycosylate other structural proteins. More long-term studies are needed to assess clinical meaning of these findings.

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REFERENCES

3. Hillman N, Herranz L, Grande C, Vaquero PM, Pallardo LF. What is the relative contribution of blood glucose levels at different time points of the day to HbA1c in Type 1 diabetes? Diabetic Medicine 2004;21:468-1470
15. Nathan DM, Turgeon, H., Regan, S., Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia 2007;50:2239-2244
APPENDIX

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**FIGURE 1. Mean glucose vs. A1c.** Mean glucose measured by the Navigator sensor over a 3-month period prior to the A1c measurement (N=75 visits from 48 subjects). Regression line calculated from a repeated measures model accounting for correlated data from the same subject.
FIGURE 2. Measures of Glycation at 3 vs. 6-month Visits. N=27 subjects with data at both visits. The ratio of mean glucose to A1c (A) and the HGI index (B) are compared at the two visits. The diagonal represents the line of identity.
FIGURE 3. Change in Mean Glucose vs. Change in A1c from 3 to 6 Months with Regression Line.