Labile A1C Is Inversely Correlated With the Hemoglobin Glycation Index in Children With Type 1 Diabetes

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OBJECTIVE — We hypothesized that labile A1C (LA1C) is directly correlated with stable A1C (SA1C) and between-patient differences in SA1C, which are independent of mean blood glucose (MBG).

RESEARCH DESIGN AND METHODS — We measured SA1C, LA1C, MBG, and a single clinic capillary glucose (CCG) from 152 pediatric patients with type 1 diabetes. Patients were grouped as high, moderate, or low glycators by hemoglobin glycation index (HGI).

RESULTS — LA1C and SA1C were correlated with CCG and MBG. LA1C was not correlated with SA1C ($r = 0.06$, $P = 0.453$). LA1C level was significantly associated with glycator group status ($P < 0.0019$) and CCG ($P < 0.0001$). Adjusted LA1C levels were highest in the low-HGI patients and lowest in the high-HGI group.

CONCLUSIONS — A conventional model of SA1C being directly correlated with LA1C concentration was not confirmed. Between-patient differences in SA1C at the same MBG may be due to complex intracellular factors influencing formation of SA1C from LA1C.

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Our team and others have described groups of diabetic patients who consistently demonstrate markedly higher (high glycators) or lower (low glycators) A1C despite both groups having similar preceding mean blood glucose (MBG) (1,2). As A1C is formed by the stable Amadori rearrangement of a precursor known as labile A1C (LA1C) (3), we hypothesized that high glycators would also have higher levels of LA1C, compared with low glycators. We tested this hypothesis in a well-characterized group of children with type 1 diabetes.

RESEARCH DESIGN AND METHODS

— Participants were patients with type 1 diabetes followed in the pediatric diabetes clinics at Children's Hospital of New Orleans. Participants had MBG calculated from data uploaded from the patient's home glucose meter and a sample drawn for A1C at each clinic visit. Visits were approximately every 3 months. A clinic capillary glucose (CCG) measurement was obtained at each visit using an Accu-Chek Inform.

A1C was assayed by a capillary isoelectric focusing method (4). LA1C was removed by incubation of 100 μl isolated erythrocytes for 6 h at 37°C in 1 ml PBS. Stable A1C (SA1C) was the level after incubation. LA1C was the difference in A1C before and after incubation. LA1C and SA1C are expressed as a percent of total HbA_0, based on peak area of absorbance at 415 nm. SA1C levels for this method were not standardized to the Diabetes Control and Complications Trial (DCCT) assay method.

Glycator status of patients was assigned by calculation of a hemoglobin glycation index (HGI) from each patient's SA1C and MBG, as previously described (1) (2). Briefly, HGI is the difference between the patient's observed and predicted SA1C. Predicted SA1C was calculated by inserting the patient's MBG into the regression equation describing the relationship between SA1C and MBG for our patient population ($SA1C = [0.031 \times MBG] + 5.4$). All patients were then ranked by HGI tertile and grouped as high-, moderate-, or low-HGI glycators (1).

Statistical methods
Assessment of the influence of HGI group on LA1C was performed with adjustment for covariates (sex, BMI, duration of diabetes, and CCG). The difference between adjusted least-square means of LA1C for the HGI group in the model was evaluated. A model was also fitted with LA1C (expressed as a percent of total A1C) as the dependent variable and HGI group, sex, BMI, duration of diabetes, and CCG as covariates.

RESULTS — Demographic and glycemic characteristics for the glycator groups are presented in Table 1. There were statistically significant differences between groups for CCG, HGI, and SA1C. MBG for the HGI groups were similar. A multiple linear regression model with LA1C as the dependent variable and HGI group, CCG, sex, BMI, and duration of diabetes as the independent variables was performed (overall $r^2 = 0.295$, $P < 0.0001$). Only HGI group ($P = 0.0019$) and CCG ($P < 0.0001$) were statistically associated with LA1C in this model.

LA1C was not correlated with SA1C ($r = 0.06$, $P < 0.45$). LA1C was correlated with both MBG ($r = 0.30$, $P < 0.0002$) and CCG ($r = 0.47$, $P < 0.0001$). SA1C was correlated with both MBG ($r = 0.62$, $P < 0.0001$) and CCG ($r = 0.38$, $P < 0.0001$).

CONCLUSIONS — In the conventionally understood model of SA1C formation, glucose enters the red blood cell (RBC) and nonenzymatically binds rapidly and reversibly to hemoglobin forming a Schiff base referred to as LA1C (3). Over longer periods of time, LA1C can undergo irreversible Amadori rearrange-

Table 1—Demographic characteristics and glycemic measurements by HGI group status

HGI group	n	Age (years)	Duration of diabetes (years)	Sex (n male/female)	HGI*	MBG (mg/dl)	CCG (mg/dl)	SA1C* (%)	Adjusted LA1C (%)	LA1C (% total A1C)
High	54	13.1 ± 3.6	5.6 ± 3.6[b]	27/27	1.85 ± 1.20[a]	195 ± 48	276 ± 106[a]	13.4 ± 1.8[a]	1.88[b]	13.6 ± 6.0[c]
Moderate	52	12.6 ± 4.1	4.7 ± 3.5[b]	25/27	−0.07 ± 0.44[b]	181 ± 31	225 ± 86[b]	11.0 ± 1.1[b]	2.33[ab]	16.5 ± 6.3[b]
Low	46	12.1 ± 3.7	3.9 ± 3.0[a]	27/19	−1.79 ± 0.74[c]	187 ± 45	221 ± 104[b]	9.5 ± 1.5[c]	2.60[a]	20.0 ± 6.6[a]

Data are means ± SD. *HGI and SA1C are different between groups due to group selection. [abc]Group values within a column having different superscripts are significantly different ($P < 0.05$) from each other.

ment to form SA1C (3). Once formed, SA1C accumulates intracellularly over the lifespan of the RBC. The model suggests that formation of SA1C is proportional to the concentration of precursor moieties, a concept supported in part by the observation that LA1C is correlated with concurrent clinic glucose level (CCG) and SA1C is correlated with MBG. Based on this basic model, we hypothesized that LA1C would be correlated with SA1C and that high glycators would have correspondingly high levels of LA1C compared with low glycators. Contrary to our expectations, we found that *1*) the concentrations of LA1C and SA1C within RBCs were not correlated and *2*) low glycators had the highest levels of LA1C adjusted for the concurrent glucose level.

Differences in SA1C between individuals and species despite similar MBG might be due to differences in intracellular glucose levels (5–8). However, if higher CCG and MBG lead to higher intracellular glucose concentrations, this does not appear to translate into higher LA1C levels for high glycators in vivo. Thus, factors in addition to intracellular glucose concentration may influence LA1C and subsequent formation of SA1C, contributing to observed differences between high and low glycators despite similar MBG. LA1C is 60 times more likely to revert back to free glucose and hemoglobin than form SA1C (9). Thus, relatively minor changes in conditions may alter subsequent formation of SA1C from LA1C. Potential altering factors may be intracellular pH, competitive binding of glucose and other metabolites, other isoforms of LA1C, and deglycating enzymes (10). Thus, intra-RBC factors may favor accumulation of LA1C over formation of SA1C in low glycators.

Although definitive evidence is not yet available, there are several potential explanations for lack of correlation between LA1C and SA1C, although both are correlated to a lesser or greater degree to CCG and MBG. Different isoforms of LA1C with different association/dissociation kinetics (11), the short time frame (minutes to hours) of LA1C formation/dissociation compared with longer formation (days to weeks) of SA1C (9), RBC longevity, oxidative status, and deglycating enzymes may all differentially influence levels of LA1C compared with SA1C. These factors potentially lead to the observed differences in proportion of LA1C to SA1C and HGI between individuals.

Low glycators are at less risk for microvascular complications than high glycators (2,12,13). It is tempting to speculate that higher LA1C could serve as a temporary intracellular storage compartment for glucose and/or some of its intracellular metabolites. Temporary sequestering of glucose or glucose metabolites as LA1C would prevent these substances from entering pathways that produce toxic metabolites when blood glucose levels are elevated.

The process by which hemoglobin and other proteins become glycated is likely more complex than conventionally described. Our findings suggest that factors in addition to simple concentration dependent kinetics play a role in the formation of SA1C and observed biological variation between high and low glycators.

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