

Labile Hemoglobin A1c is Inversely Correlated with the Hemoglobin Glycation Index in Children with Type 1 Diabetes

Running Title: Labile HbA1c and HGI

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Objective: We hypothesized that labile HbA1c (LHbA1c), is directly correlated with stable HbA1c (SHbA1c) and between-patient differences in SHbA1c which are independent of mean blood glucose (MBG).

Research design and method: We measured SHbA1c, LHbA1c, MBG and a single clinic capillary glucose (CCG) from 152 pediatric patients with type 1 diabetes. Patients were grouped as High, Moderate, or Low glycaters by Hemoglobin Glycation Index.

Results: LHbA1c and SHbA1c were correlated with CCG and MBG. LHbA1c was not correlated with SHbA1c ($r=0.06$, $p=0.453$). LHbA1c level was significantly associated with glycation group status ($p<0.0019$) and CCG ($p<0.0001$). Adjusted LHbA1c levels were highest in the Low HGI patients and lowest in the High HGI group.

Conclusion: A conventional model of SHbA1c being directly correlated with LHbA1c concentration was not confirmed. Between patient differences in SHbA1c at the same MBG may be due to complex intracellular factors influencing formation of SHbA1c from LHbA1c.

Our team and others have described groups of diabetes patients who consistently demonstrate markedly higher (High Glycators) or lower (Low Glycators) HbA1c despite both groups having similar preceding mean blood glucose (MBG)(1)(2). As HbA1c is formed by the stable Amadori rearrangement of a precursor known as labile HbA1c (LHbA1c)(3), we hypothesized that High Glycators would also have higher levels of LHbA1c, compared to 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 HbA1c at each clinic visit. Visits were approximately every three months. A clinic capillary glucose (CCG) measurement was obtained at each visit using an Accu-Chek Inform.

HbA1c was assayed by a capillary isoelectric focusing method(4). LHbA1c was removed by incubation of 100 μ l of isolated erythrocytes for 6 h at 37°C in 1 ml of phosphate buffered saline. Stable HbA1c (SHbA1c) was the level after incubation. LHbA1c was the difference in HbA1c before and after incubation. LHbA1c and SHbA1c are expressed as a percent of total HbA₀ based on peak area of absorbance at 415 nm. SHbA1c levels for this method were not standardized to the DCCT assay method.

Glycator status of patients was assigned by calculation of a Hemoglobin Glycation Index (HGI) from each patient's SHbA1c and MBG as previously described(1)(2). Briefly, HGI is the difference between the patient's observed and predicted SHbA1c. Predicted SHbA1c was calculated

by inserting the patient's MBG into the regression equation describing the relationship between SHbA1c and MBG for our patient population ($\text{SHbA1c} = (0.031 \times \text{MBG}) + 5.4$). 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 LHbA1c was performed with adjustment for covariates (sex, BMI, duration of diabetes, CCG). The difference between adjusted least square means of LHbA1c for HGI group in the model was evaluated. A model was also fitted with LHbA1c (expressed as a percent of total HbA1c) 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 SHbA1c. MBG for the HGI groups were similar. A multiple linear regression model with LHbA1c as the dependent variable and HGI group, CCG, gender, 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 LHbA1c in this model.

LHbA1c was not correlated with SHbA1c ($r=0.06$, $p<0.45$). LHbA1c was correlated with both MBG ($r=0.30$, $p<0.0002$) and CCG ($r=0.47$, $p<0.0001$). SHbA1c 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 SHbA1c formation: glucose enters the RBC, nonenzymatically binds rapidly and

reversibly to hemoglobin forming a Schiff base referred to as LHbA1c(3). Over longer periods of time LHbA1c can undergo irreversible Amadori rearrangement to form SHbA1c(3). Once formed SHbA1c accumulates intracellularly over the lifespan of the RBC. The model suggests that formation of SHbA1c is proportional to the concentration of precursor moieties, a concept supported in part by the observation that LHbA1c is correlated with concurrent clinic glucose level (CCG), and SHbA1c is correlated with MBG. Based on this basic model we hypothesized that LHbA1c would be correlated with SHbA1c, and that High Glycators would have correspondingly high levels of LHbA1c compared to Low Glycators. Contrary to our expectations we found that 1) the concentrations of LHbA1c and SHbA1c within RBCs were not correlated and 2) Low glycators had the highest levels of LHbA1c adjusted for the concurrent glucose level.

Differences in SHbA1c between individuals and species despite similar MBG might be due to differences in intracellular glucose levels(5)(6)(7)(8). However, if higher CCG and MBG lead to higher intracellular glucose concentrations this does not appear to translate into higher LHbA1c levels for High Glycators in vivo. Thus factors in addition to intracellular glucose concentration may influence LHbA1c and subsequent formation of SHbA1c, contributing to observed differences between High and Low Glycators despite similar MBG. LHbA1c is 60 times more likely to revert back to free glucose and hemoglobin than form SHbA1c(9). Thus relatively minor changes in conditions may alter subsequent formation of SHbA1c from LHbA1c. Potential altering factors may be intracellular pH, competitive binding of glucose and other

metabolites, other isoforms of LHbA1c, and deglycating enzymes(10). Thus intra-RBC factors may favor accumulation of LHbA1c over formation of SHbA1c in Low Glycators.

Although definitive evidence is not yet available, there are several potential explanations for lack of correlation between LHbA1c and SHbA1c, although both are correlated to a lesser or greater degree to CCG and MBG. Different isoforms of LHbA1c with different association/dissociation kinetics(11), the short time frame (minutes to hours) of LHbA1c formation/dissociation compared to longer formation (days to weeks) of SHbA1c (9), RBC longevity, oxidative status, deglycating enzymes may all differentially influence levels of LHbA1c compared to SHbA1c. These factors potentially lead to the observed differences in proportion of LHbA1c to SHbA1c 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 LHbA1c 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 LHbA1c 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 glycosylated 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 SHbA1c and observed biological variation between High and Low Glycators.

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

HGI Group	n	Age (years)	Duration of Diabetes (years)	Gender (#M/F)	HGI*	MBG (mg/dL)	CCG (mg/dL)	SHbA_{1c}* (%)	Adjusted LHbA_{1c} (%)	LHbA_{1c} (% Total HbA_{1c})
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

*HGI and SHbA_{1c} are different between groups due to group selection

^{abc} Group values (means±SD) within a column having different superscripts are significantly different (p<0.05) from each other