Implications of the Hemoglobin Glycation Index on the Diagnosis of Prediabetes and Diabetes

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Objective: Fasting plasma glucose (FPG), 2-hour plasma glucose (2hPG) from a 75-g oral glucose tolerance test (OGTT) and glycated hemoglobin (HbA1c) can lead to different results when diagnosing prediabetes and diabetes. The Hemoglobin Glycation Index (HGI) quantifies the interindividual variation in glycation resulting in discrepancies between FPG and HbA1c. We used data from the Vitamin D and Type 2 Diabetes (D2d) study to calculate HGI, to identify HGI-associated variables, and to determine how HGI affects prediabetes and diabetes diagnosis.

Measurements: A linear regression equation [HbA1c (%) = 0.0164 × FPG (mg/dL) + 4.2] was derived using the screening cohort (n = 6829) and applied to calculate predicted HbA1c. This was subtracted from the observed HbA1c to determine HGI in the baseline cohort with 2hPG data (n = 3945). Baseline variables plus prediabetes and diabetes diagnosis by FPG, HbA1c, and 2hPG were compared among low, moderate, and high HGI subgroups.

Results: The proportion of women and Black/African American individuals increased from low to high HGI subgroups. Mean FPG decreased and mean HbA1c increased from low to high HGI subgroups, consistent with the HGI calculation; however, mean 2hPG was not significantly different among HGI subgroups.

Conclusions: High HGI was associated with Black race and female sex as reported previously. The observation that 2hPG was not different across HGI subgroups suggests that variation in postprandial glucose is not a significant source of population variation in HGI. Exclusive use of HbA1c for diagnosis will classify more Black individuals and women as having prediabetes compared with using FPG or 2hPG. (*J Clin Endocrinol Metab* 105: 1–9, 2020)

Key Words: hemoglobin glycation, prediabetes, diagnosis, type 2, oral glucose tolerance test; observational study

Abbreviations: 2hPG, 2-hour plasma glucose;ANOVA, analysis of variance;BMI, body mass index;D2d, Vitamin D and Type 2 Diabetes;FPG, fasting plasma glucose; HbA1c, glycated hemoglobin A1c;HGI, Hemoglobin Glycation Index;MBG, mean blood glucose;NGSP, National Glycohemoglobin Standardization Program;OGTT, oral glucose tolerance test.

Fasting plasma glucose (FPG), 2-hour plasma glucose (2hPG) from a 75-g oral glucose tolerance test (OGTT), and glycated hemoglobin A1c (HbA1c) are each used clinically for the diagnosis and management of prediabetes and diabetes. While FPG and 2hPG are specific measures of blood glucose concentration, HbA1c reflects more than just glycemia. Research has shown that diabetes classification based on these 3 tests can differ markedly in human populations (1, 2). One major cause of differences in the diagnostic specificity of each test is interindividual variation in the quantitative relationship between HbA1c and plasma glucose concentration (3, 4). For example, Blacks/African Americans tend to have higher HbA1c levels than whites with similar blood glucose concentrations (3, 5, 6). It has been suggested that interindividual differences in red blood cell life span or other biological factors can influence HbA1c levels independent of the effect of plasma glucose concentration (7). Variation in the quantitative relationship between HbA1c and blood glucose has important clinical implications for how HbA1c, FPG, and 2hPG should be used in the diagnosis of prediabetes and diabetes and for prediction of diabetes complications (8).

The Hemoglobin Glycation Index (HGI) is a biomarker of population variation in HbA1c due to factors other than blood glucose concentration (9). HGI quantifies the magnitude and direction of interindividual variation in HbA1c based on the difference between an observed (measured) HbA1c and a predicted HbA1c. The predicted HbA1c is calculated by inserting an individual's FPG into a linear regression equation that describes the quantitative relationship between FPG and HbA1c in a study population (10–12). Because of how HGI is calculated, people with a low HGI phenotype have higher FPG than people with a high HGI phenotype and similar HbA1c. Likewise, people with a high HGI phenotype have higher HbA1c than people with a low HGI phenotype and similar FPG. Thus, one might predict that disproportionately more people with low HGI would be classified as having prediabetes or diabetes based on FPG; while disproportionately more people with high HGI would be classified as having prediabetes or diabetes based on HbA1c. It is less clear whether variation in postprandial glucose is associated with HGI, or whether the proportion of diagnoses of prediabetes or diabetes based on 2hPG differs among HGI subgroups (13).

It has been previously reported that patients with type 1 or type 2 diabetes and high HGI calculated using either FPG or mean blood glucose (MBG, from patient meters or 1-day profile sets collected before and after meals) were at greater risk for microvascular complication hypoglycemia and cardiovascular disease (10, 11, 14–17). Moreover, higher HGI in people without diabetes has been associated with increased coronary artery calcification (18), carotid atherosclerosis (19), hepatic steatosis (20), kidney dysfunction (21), and inflammation (12, 19, 22). Other investigators have suggested that because HGI is strongly associated with HbA1c, it cannot be an independent predictor of microvascular complications (23). The degree to which HGI represents a more discriminating biomarker of risk for complications compared with HbA1c alone remains to be established.

The Vitamin D and Type 2 Diabetes (D2d) study is a large prospective clinical trial designed to investigate the effect of vitamin D supplementation on prevention of diabetes in people with prediabetes (24). This analysis used D2d study data (individuals with complete screening and baseline visit data) to calculate HGI, to identify demographic and clinical variables associated with HGI, and to determine how HGI is associated with prediabetes and diabetes diagnosis. These analyses will lay the groundwork for future longitudinal analyses of prospective diabetes outcomes over time in participants with low and high HGI.

Materials and Methods

Overview of the D2d study

D2d is a US-based clinical trial conducted at 22 sites to evaluate oral administration of vitamin D compared with placebo for prevention of diabetes in people at high risk for diabetes. The experimental design of D2d has been published (24). The study was approved and monitored by an independent data and safety monitoring board and the institutional review board of each collaborating site.

Study population

Target participants were adults at high risk for diabetes (24). At the screening visit, glycemic criteria for prediabetes were preliminarily evaluated by measuring FPG and HbA1c either in a local laboratory or at the D2d central laboratory. If participants had FPG and HbA1c values that suggested they might qualify for the study (cutoffs varied slightly between centers but generally required FPG and HbA1c to be in the prediabetes range), participants were invited to proceed to a baseline visit on the same day or on another day. At the baseline visit, a 75-g OGTT was performed, and fasting FPG, 2hPG, and HbA1c were measured at the D2d central laboratory to determine final eligibility. Participants ultimately randomized had to meet at least 2 criteria for prediabetes as established by the American Diabetes Association (ADA) in 2010 (25): FPG: 100 to 125 mg/dL (5.6–6.9 mmol/L); 2hPG: 140 to 199 mg/dL (7.8–11.0 mmol/L) in a 75-g OGTT; and/or HbA1c: 5.7% to 6.4% (39–46 mmol/mol).

Other entry criteria included age ≥ 30 years (≥ 25 years for American Indians, Alaska Natives, Native Hawaiians, or other Pacific Islanders) and body mass index (BMI) of 24 to 42 kg/m^2 (22.5 to 42 kg/m^2 for Asians). For this current analysis, data from the whole screening cohort were used to derive a linear regression equation of HbA1c versus FPG and to derive HGI tertile cutpoints. HbA1c and FPG from the baseline visit were then entered into the regression equation to calculate HGI and to divide participants in the baseline cohort into HGI subgroups based on the screening HGI tertile cutpoints.

Laboratory methods

At the baseline visit, blood samples for glucose analysis were collected in sodium fluoride tubes, refrigerated, and centrifuged within 45 minutes of collection. Plasma from these samples was stored at –70°C and shipped frozen to the D2d central laboratory. These samples were analyzed for glucose using a hexokinase method (Roche Glucose HK Gen.3 on the Cobas Integra 400 or Cobas c311 analyzer, Roche Diagnostics, Indianapolis, IN), standardized against isotope dilution mass spectrometry (ID/MS). Blood samples for HbA1c analysis were collected in EDTA tubes and stored refrigerated until they were shipped refrigerated. HbA1c was measured within 7 days of collection using an ion-exchange high-performance liquid chromatography (HPLC) method (Tosoh G8, Tosoh Bioscience, South San Francisco, CA). This method is certified by the National Glycohemoglobin Standardization Program (NGSP), and the D2d Central Laboratory is certified by NGSP as a Level I Laboratory with documented traceability to the Diabetes Control and Complications Trial Reference Method (26). At the beginning of the study, approximately half of the screening samples were analyzed at each local site by a variety of methods for glucose and HbA1c.

Calculation of hemoglobin glycation index (HGI)

A linear regression equation of HbA1c versus FPG was derived using all data from the screening cohort with the exception of 2 outliers for FPG and HbA1c that were not clinically plausible (n = 6829). Due to the structure of the D2d study, the larger screening cohort was used to derive the regression equation because the initial screening process limited the range of FPG and HbA1c values in the subsequent baseline cohort. HGI was then calculated as the difference between the observed (measured) HbA1c and a predicted HbA1c derived by inserting a date-matched blood glucose measurement into the linear regression equation (see below) and applied to the subset of participants in the baseline cohort with complete values for FPG, HbA1c, and 2hPG (n = 3945).

Data analyses

The baseline cohort was divided into low, moderate, and high HGI subgroups based on screening cohort HGI tertile cutpoints. Available baseline variables that have been shown to be important in previous HGI studies (age, BMI, sex, race, and ethnicity) were compared among the 3 HGI subgroups. Race was defined as Black/African American or non-African American; ethnicity was classified as Hispanic/Latino or non-Hispanic/Latino. Analysis

of variance (ANOVA) for continuous variables (age, BMI) and chi-square analysis for categorical variables (sex, race, ethnicity) were used to determine differences among HGI groups. Comparisons of glycemia by FPG, HbA1c, and 2hPG were made among the 3 HGI subgroups via ANOVA. Statistical significance was determined using two-tailed tests with a significance level of $P < 0.05$. All analyses were conducted using R version 3.4.2.

Results

Characteristics of screening and baseline cohort

Characteristics of the screening and baseline cohort are presented in Table 1 and the groups were noted to be similar overall.

HGI cutpoints

Based on data from the screening visit, a linear regression equation [HbA1c (%) = 0.0164 × FPG (mg/dL) + 4.2] was established and then applied to calculate predicted HbA1c and HGI for each participant in the baseline cohort (Fig. 1). Cutpoints for assignment to either the low (≤ –0.153), moderate (> –0.153 to 0.130), or high (> 0.130) HGI subgroup were established based on HGI tertiles established using the screening data and then applied to the baseline cohort.

Association between HGI cutpoints and characteristics of baseline cohort

The proportions of women and Blacks/African Americans increased across subgroups of HGI from low to high HGI ($P < 0.001$). Age, ethnicity, and BMI were not different among HGI subgroups ($P = 0.62$, $P = 0.06$, and $P = 0.18$, respectively). Mean FPG decreased and HbA1c increased going from low to high HGI subgroups, consistent with how HGI is calculated. Mean 2hPG was not significantly different among HGI subgroups ($P = 0.80$).

Association between HGI cutpoints and diagnosis of prediabetes and diabetes

Over 80% of low HGI participants had FPG in the prediabetes range compared with just over 50% of high HGI participants ($P < 0.001$) (Table 2). In contrast, over 90% of high HGI participants had HbA1c in the prediabetes range compared with less than 60% of low HGI participants ($P < 0.001$). There was no difference in the prevalence of people with 2hPG in the prediabetes range among HGI subgroups ($P = 0.29$). Fig. 2 uses the values from Table 2 to show that the combined diagnoses of prediabetes and diabetes differed among HGI

Table 1.   Characteristics of the Screening and Baseline Cohort

Variable	Screening N = 6829	Baseline N = 3945	Low HGI N = 1495	Moderate HGI N = 1419	High HGI N = 1031	P Value[a]
HGI, mean (SD)	0.00 (0.38)	-0.06 (0.29)	-0.35 (0.17)	-0.01 (0.08)	0.30 (0.13)	<0.001
Age (y), mean (SD)	59.4 (10.2)	59.4 (10.2)	59.4 (10.4)	59.6 (10.2)	59.3 (9.8)	0.62
Sex, n (%)						<0.001
Female	3371 (49.4)	1799 (45.6)	596 (39.9)	668 (47.1)	535 (51.9)	
Male	3458 (50.6)	2146 (54.4)	899 (60.1)	751 (52.9)	496 (48.1)	
Race, n (%)						<0.001
Black/African American	1746 (25.6)	1008 (25.6)	190 (12.7)	347 (24.5)	471 (45.7)	
Non-African American	5083 (74.4)	2937 (74.4)	1305 (87.3)	1072 (75.5)	560 (54.4)	
Ethnicity, n (%)						0.06
Hispanic	744 (10.9)	382 (9.7)	124 (8.3)	154 (10.9)	104 (10.1)	
Non-Hispanic	6085 (89.1)	3563 (90.3)	1371 (91.7)	1265 (89.1)	927 (89.9)	
BMI (kg/m^2), mean (SD)	31.9 (4.5)	31.9 (4.5)	31.9 (4.4)	31.7 (4.5)	32.0 (4.6)	0.18
FPG (mg/dL), mean (SD)	103.6 (14.6)	106.7 (10.7)	111.7 (10.9)	105.5 (9.2)	101.1 (9.1)	<0.001
HbA1c (%), mean (SD)	5.9 (0.5)	5.9 (0.4)	5.7 (0.2)	5.9 (0.2)	6.1 (0.2)	<0.001
2hPG (mg/dL), mean (SD)	N/A	137.9 (44.9)	137.6 (45.7)	137.6 (44.7)	138.7 (44.1)	0.80

The Baseline cohort was divided into Low, Moderate, and High subgroups.
Abbreviations: 2hPG, 2-hour plasma glucose; ANOVA, analysis of variance; BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin A1c; HGI, Hemoglobin Glycation Index; SD, standard deviation.
[a]ANOVA for continuous variables and chi-square analysis for categorical variables were used to determine differences among low, moderate, and high HGI groups
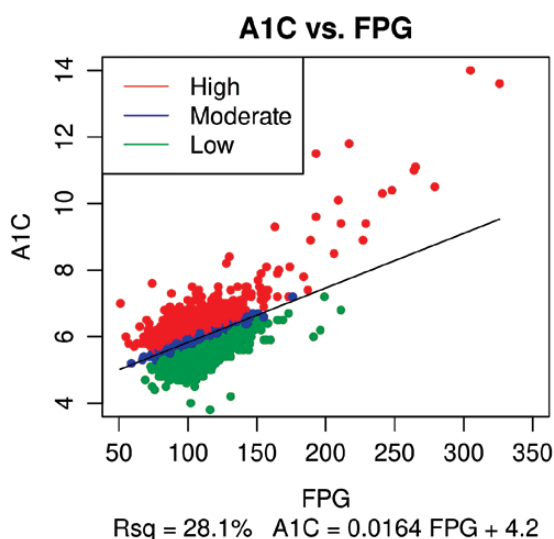

Figure 1. Diagram of Linear Regression Equation Based on the Entire Screening Cohort (N = 6830). Red indicates high HGI, blue indicates moderate HGI, and green indicates low HGI.

subgroups based on FPG (≥ 100 mg/dL) and HbA1c (≥ 5.7% / 39 mmol/mol) but not on the results of the 2hPG level (≥ 140 mg/dL).

Discussion

HGI is the residual derived using a blood glucose versus HbA1c linear regression equation from a reference population; whereas, the glycosylation (glycation) gap is a similar residual derived using fructosamine versus HbA1c. Dr. Hempe and colleagues first proposed the HGI in 2002 (14) and the glycosylation gap was proposed by Cohen et al in 2003 (15). In the last 2 years there have been at least 20 reports looking at relationships between HGI and chronic disease in both nondiabetic and diabetic study populations. People with high HGI exhibit similar traits whether the glucose component of the regression equation is estimated using FPG, MBG, or fructosamine (15, 27, 28) and regardless of whether MBG was calculated using patient glucose meter data (14, 16), continuous glucose monitoring (17), or 7-point glucose profile sets (9). Thus, not sampling blood glucose in the postprandial period does not appear to affect HGI. The observation of phenotypic consistency, regardless of how blood glucose status is measured, strongly suggests that FPG, MBG, and fructosamine all similarly reflect the most important

Table 2.   Prediabetes and Diabetes Diagnosis by HGI Subgroup

	Total N = 3945	Low HGI N = 1495	Moderate HGI N = 1419	High HGI N = 1031	P Value
FPG, n (%)					<0.001
Normal	965 (24.5)	133 (8.9)	370 (26.1)	462 (44.8)	
Prediabetes	2819 (71.5)	1242 (83.1)	1015 (71.5)	562 (54.5)	
Diabetes	161 (4.0)	120 (8.0)	34 (2.4)	7 (0.7)	
HbA1c, n (%)					<0.001
Normal	698 (17.7)	641 (42.9)	56 (3.9)	1 (0.1)	
Prediabetes	3197 (81.0)	852 (57.0)	1362 (96.0)	983 (95.3)	
Diabetes	50 (1.3)	2 (0.1)	1 (0.1)	47 (4.6)	
2hPG, n (%)					0.29
Normal	2226 (56.4)	853 (57.1)	806 (56.8)	567 (55.0)	
Prediabetes	1360 (34.5)	497 (33.2)	482 (34.0)	381 (37.0)	
Diabetes	359 (9.1)	145 (9.7)	131 (9.2)	83 (8.0)	

Abbreviations: 2hPG, 2-hour plasma glucose; BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin A1c; HGI, Hemoglobin Glycation Index.
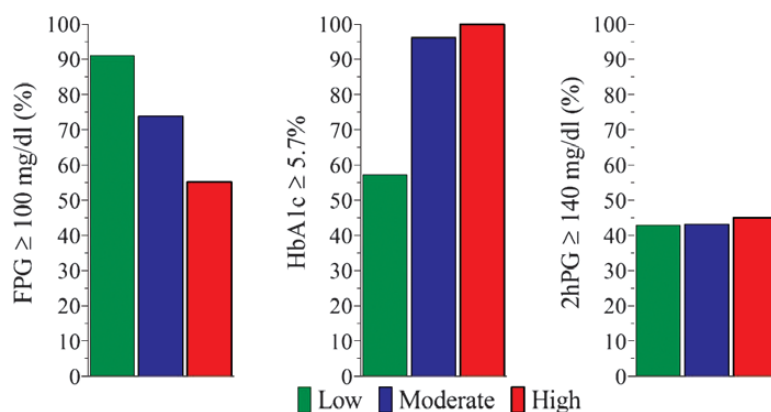

Figure 2. Prevalence of combined diagnoses of prediabetes and diabetes based on FPG, HbA1c, and 2hPG by HGI subgroups.

aspects of person-to-person variation in blood glucose that affect the quantitative relationship between blood glucose and HbA1c measured by HGI. If using MBG, fructosamine, and FPG to calculate HGI all give similar results, then FPG will be the metric of choice because it is simpler, less expensive, and more widely used, especially in developing countries.

Our screening and baseline data from the D2d study identified differences among HGI subgroups in race and sex, some of which have been reported in previous studies. Our analysis showed no difference in BMI among HGI subgroups. This observation is similar to what has been reported in previous analyses of HGI and BMI in people with diabetes (10, 29–33). In contrast, multiple studies have reported that BMI is higher in people without diabetes with high HGI (18, 19, 22). Whether low or high HGI predicts who will develop type 2 diabetes is a question we plan to examine using longitudinal data that recently became available in the D2d study.

Our analysis found that the high HGI subgroup included disproportionately more Blacks/African Americans and women. Evidence of racial variation in

HGI confirms multiple reports which have shown that Blacks/African-Americans tend to have higher HbA1c compared with whites with similar blood glucose levels in both nondiabetic and diabetic study populations (3, 5, 6, 22, 34, 35). The high HGI subgroup in the D2d study also contained disproportionately more women as previously observed in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial (10). Evidence of a relationship between sex and HGI has not been consistent across studies, however, and thus whether or not HGI is associated with sex remains equivocal. The etiology of race or sex differences in HGI could be related to heritable differences in erythrocyte lifespan (36), glucose transport (37), or the fact that both women and people of more recent African descent tend to have lower than average hemoglobin levels (6, 38, 39).

As predicted, the low HGI subgroup had disproportionately more individuals diagnosed with prediabetes or diabetes based on FPG alone and the high HGI subgroup had disproportionately more individuals diagnosed with prediabetes or diabetes based on HbA1c alone (Table 2 and Fig. 2). We acknowledge that these findings were the

result of how HGI is calculated. However, the third metric of glycemic status, 2hPG, was not significantly different among HGI subgroups. Other investigators (40, 41) have hypothesized that biological variation in HGI might be an analytical artifact caused by person-to-person variation in postprandial glucose excursions that are not detected when FPG is used to calculate HGI. This hypothesis is supported by the results of a study in people without diabetes, with prediabetes, and with treatment-naïve diabetes, conducted by Ahn et al (12) who reported higher 2hPG levels in high-HGI study participants. In contrast, our results agree with those of Marini et al (19), who reported no difference in 2hPG between HGI subgroups in a study population without diabetes or with prediabetes, potentially indicating minimal contribution of postprandial glucose excursions to the biological variation in HGI. This would also make sense, since people without diabetes spend very little time in the postprandial state.

If 2hPG is not significantly different among HGI subgroups, we hypothesize that HGI may be a useful biomarker whenever there is a discrepancy between HbA1c and FPG. In people with a low HGI phenotype who should have lower HbA1c levels than their high HGI counterparts, an elevated HbA1c in the prediabetes or diabetes range may be demonstrating postprandial glucose intolerance/insulin insufficiency and would require an OGTT to further determine blood glucose control. Alternatively, in people with a high HGI phenotype who should have lower FPG levels than their low HGI counterparts, an elevated FPG in the prediabetes or diabetes range may be demonstrating hyperglucagonemia, (42) for example, and an OGTT could help further characterize any additional postprandial glucose intolerance. In addition, use of an OGTT for diabetes screening may be preferred to prevent overdiagnosis based on HbA1c alone in those with high HGI or overdiagnosis based on FPG in those with low HGI. However, the value of HGI in predicting the future incidence of diabetes in those with prediabetes cannot be determined from this cross-sectional analysis and requires longitudinal follow-up. With the diabetes outcomes now reported in the D2d study, we will have additional information to determine which HGI subgroup is more likely to progress to diabetes. Additional studies are needed to investigate the value of HGI in clinical practice to help determine the most appropriate screening method for prediabetes and diabetes in certain populations.

Our study has several strengths, including a large sample size, extensive phenotyping of a large number of people at risk for diabetes, and extensive evaluation of glycemic status (including FPG, HbA1c, and 2hPG). Our approach was consistent with several other studies using HGI. Our study did have some limitations. A small subset of the screening tests used in the HGI regression equation were analyzed at different local laboratories introducing possible interlaboratory variation; however, about half of the screening labs were analyzed in the central lab and all local labs had NGSP certification for HbA1c. In addition, we used the screening population to derive the equation and then applied it to the baseline population. Although the baseline cohort had less glycemic variability because participants had to pass the screening glycemic criteria to attend a baseline visit, the demographic characteristics of the 2 populations were similar. Because fructosamine was not measured in this study, the glycation gap could not be calculated and compared with results using HGI. Limitations of HGI as a clinical tool include the lack of a single regression equation that can be used to calculate HGI for every population.

Future work could examine data from continuous glucose monitoring during the 3 months prior to the HbA1c measurement as a direct measure of mean glycemia, although this method would be difficult to implement in large clinical trials, such as the D2d study. Moreover, similar interindividual variation was seen between HbA1c and mean glucose from continuous glucose monitoring data in the development of the Glucose Management Indicator (GMI), an estimated HbA1c level derived from a regression equation of mean glucose and measured HbA1c in diabetes patients (42).

In conclusion, evidence of clinically significant interindividual variation in the relationship between blood glucose and HbA1c complicates the use of any single test for the diagnosis of prediabetes and diabetes. HGI classification may be affected by race and sex. Moreover, blood glucose control as measured by FPG and HbA1c may be influenced by HGI classification, but 2hPG may help better characterize blood glucose control in certain populations. Our results suggest that normative values and longitudinal follow-up using HGI should be further evaluated, since HGI may serve as an additional variable when deciding which tests to use in which patients for diagnosing prediabetes and diabetes.

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