Potential Clinical Error Arising From Use of HbA_{1c} in Diabetes: Effects of the Glycation Gap

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ABSTRACT The glycation gap (GGap) and the similar hemoglobin glycation index (HGI) define consistent differences between glycated hemoglobin and actual glycemia derived from fructosamine or mean blood glucose, respectively. Such a disparity may be found in a substantial proportion of people with diabetes, being >1 U of glycated HbA_{1c}% or 7.2 mmol/mol in almost 40% of estimations. In this review we define these indices and explain how they can be calculated and that they are not spurious, being consistent in individuals over time. We evaluate the evidence that GGap and HGI are associated with variation in risk of complications and mortality and demonstrate the potential for clinical error in the unquestioning use of HbA_{1c}. We explore the underlying etiology of the variation of HbA_{1c} from mean glucose in blood plasma, including the potential role of enzymatic deglycation of hemoglobin by fructosamine-3-kinase. We conclude that measurement of GGap and HGI are important to diabetes clinicians and their patients in individualization of therapy and the avoidance of harm arising from consequent inappropriate assessment of glycemia and use of therapies. ***(Endocrine Reviews 40: 988 – 999, 2019)***

HbA_{1c} has become the *sine qua non* of diagnosis and of clinical study outcome measures with few diabetes professionals questioning its apparent validity (1). Nevertheless, a historical perspective shows that, in fact, many doubts have been aired during the last 30 years about such an unquestioning assumption [as recently emphasized by Cohen *et al.* (2)]. Although for most patients with (or suspected of having) diabetes mellitus the use of HbA_{1c} provides a tool that yields helpful guidance in diagnosis and treatment, there is an increasing body of evidence that for a substantial minority a more nuanced and individualized approach is appropriate (3). In short, for these patients the blunt use of HbA_{1c} to guide treatment and diagnosis may lead to significant clinical errors. It is therefore important for those involved in the care of patients to understand the impact of the glycation gap (GGap) and its sister the hemoglobin glycation index (HGI) on the validity of HbA_{1c} measurements (4–6).

Nonenzymatic Glycation of Blood Proteins and Its Use in Estimating Average Glycemia

Hyperglycemia of diabetes is associated with increased glycation of free amino groups in proteins. Protein glycation is a key factor leading to vascular complications and, furthermore, when occurring in erythrocyte proteins, it provides the widely used index of average glycemia, HbA_{1c} or glycohemoglobin (GHb) (7). Glycation occurs as a result of the well-known reaction of carbohydrate moieties with amino groups of proteins known since 1910 as the Maillard reaction or, more specifically when involving glucose, as the Schiff reaction. The aldimine product of the Schiff reaction undergoes slow but reversible rearrangement to the Amadori (ketoamine) product. The ketoamine is then slowly converted to advanced glycation end products that comprise a wide range of chemical

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ESSENTIAL POINTS

- The glycation gap (GGap) and hemoglobin glycation index (HGI) show an individually consistent difference between HbA_{1c} and other measures of mean glycemia
- GGap/HGI may be found in a substantial proportion (almost 40%) of people with diabetes
- We define GGap and its calculation and show that it is consistent and not spurious
- We evaluate the considerable evidence that GGap and HGI are associated with variation in risk of complications and mortality associated with diabetes
- We explore the etiology of GGap/HGI, including the potential role of a deglycating enzyme FN3K
- We conclude that the measurement of GGap and HGI are important to diabetes clinicians and their patients in individualization of therapy and the avoidance of harm arising from consequent inappropriate assessment of glycemia and use of therapies

moieties that contribute to the development of complications of diabetes [for a review, see Zhang *et al.*, 2009 (8)].

Accurate quantification of glycemia with reliable and practicable tests was historically a challenge. Before the development of accurate point-of-care devices, blood glucose measurement and monitoring depended on inaccurate clinic or home based "stick" testing of blood glucose or else necessarily infrequent laboratory measurements.

As early as 1964, an unusual abnormal hemoglobin, HbA_{1c} ("blocked" at the N terminus of the β-chain), was identified chromatographically (9). By the mid-1970s this was shown to be a "glycosylated" variant that was elevated by approximately twofold in patients with diabetes along with other variants (HbA_{1a} and HbA_{1b}) compared with those without diabetes (10, 11). HbA_{1c} was later shown to be the major subcomponent of total glycated hemoglobin, resulting from nonenzymatic glycation at the N-terminal valine of the β-chain of hemoglobin A; other glycations by glycolytic intermediates fructose-1,6-bisphosphate and glucose-6-phosphate produce variants such as HbA_{1a} and Hb_{1b}, and glycation at amino groups of intrachain lysines also occur but do not affect the chromatographic mobility of hemoglobin. Originally referred to as glycosylated hemoglobin, the concept of glycation, a nonenzymatic reaction between glucose and free amino groups on proteins, was developed to distinguish this process from the posttranslational glycosylation of proteins, and the use of the name "glycated hemoglobin" was proposed in 1983 (12). As described above, glycation results from initial reversible reactions, with the Schiff/Maillard reaction producing an aldimine followed by a further Amadori rearrangement to a more stable glycated ketoamine (proteins thus containing fructosyl-lysine or fructosyl-(N-terminal) amino acids). Subsequently, clinical studies confirmed that HbA_{1c} could be used as a measure of glycemic control (13), its assays are now standardized (14), and currently glycated HbA_{1c} is considered the gold standard measure of glycemia during the preceding 3 months, closely associated with key microvascular complications in diabetes, proven risk reduction in complications with improvement in HbA_{1c} toward the normal nondiabetic range, and more recently implemented internationally in the diagnosis of diabetes mellitus (1, 15, 16).

However, a similar nonenzymatic glycation process occurs extracellularly with plasma proteins, predominantly albumin. Fructosamine is a marker derived from all ketoamine products occurring as a result of glycation of serum proteins and is measured by the nitroblue tetrazolium assay. Fructosamine has been proven to be as reliable an indicator of glycemic control as HbA_{1c}, representing glycemia during a shorter duration (because of the shorter half-life of serum proteins) than reflected by HbA_{1c}, and it is associated with microvascular complications in diabetes similar to HbA_{1c} (17, 18). Fructosamine as a measure of glycemic control has been validated against glycated HbA_{1c} and blood glucose (19, 20). Newer assays for fructosamine estimation are based on more specific enzymatic ketoamine oxidation compared with nitroblue tetrazolium reduction (which is subject to interference by endogenous reducing substances and other factors), although the two assays correlate closely (21). Direct assay of glycated albumin, reflecting glycemic control over weeks, has been used in some countries, has gained importance in glycemic monitoring, and is associated with microvascular complications in diabetes (17, 22)

Limitations to the Clinical Utility of HbA_{1c}—Consequences of GGap and HGI

Current strategies in the management of glycemia in diabetes rely heavily on HbA_{1c}. Despite standardization of assays, discrepancy between HbA_{1c} and other assessments of glycemia is well reported and may affect accurate interpretation of glycemic control and its management (3–6, 23, 24). A variety of erythrocytic factors that impact red cell lifespan or turnover and the glucose gradient across the red cell membrane are

known to affect HbA_{1c} independently of glycemia (25, 26). Recent changes in glycemic control are possibly overrepresented in HbA_{1c}; that is, HbA_{1c} does not reflect blood glucose levels equally during the previous 120 days. HbA_{1c} represents the net effect of several mechanisms, which may shift its direct glycation relationship with overall levels of glycemia. Various studies have calculated the deviation of HbA_{1c} co-utilizing either fructosamine or blood glucose data referred to as GGap (deviation of glycated HbA_{1c} from serum fructosamine-predicted HbA_{1c}) or HGI [discrepancy between HbA_{1c} and a predicted HbA_{1c} from date-matched mean blood glucose (MBG) estimations] (4–6, 27–29). HbA_{1c} could systematically deviate from glycemia as a result of elements that influence glycation within the red blood cells such that the HbA_{1c} might be lower (a negative GGap or low HGI implies a lower net rate of glycation) or higher (positive GGap or higher HGI, implying a higher rate of net glycation) than might be expected.

Definition and Calculation of GGap and HGI

Cohen *et al.* (5) calculated GGap as the difference between measured HbA_{1c} and the HbA_{1c} predicted from fructosamine based on the population regression of HbA_{1c} on fructosamine. Hempe *et al.* calculated the HGI as the difference between the measured HbA_{1c} and the predicted HbA_{1c} derived from date-matched MBG estimations by regression (6). Similar methodologies were used by others whereby GGap was calculated based on the regression of HbA_{1c} (y) vs fructosamine (x), and HGI was calculated based on regression of HbA_{1c} (y) vs MBG (x) (27–29). Statistically, the calculated GGap (or HGI) would thus be a linear function of HbA_{1c} and fructosamine (or MBG); GGap or HGI thus calculated would be significantly correlated with HbA_{1c}, and hence it would be difficult to dissect the association with complications independent of HbA_{1c}. Furthermore, the fructosamine-derived HbA_{1c} in GGap, or MBG-derived HbA_{1c} in HGI, would not be independent of HbA_{1c}, and thus it would be statistically spurious to include HbA_{1c} in an analysis along with GGap or HGI (30).

In a previous study to assess the clinical impact of variability in HbA_{1c}, we calculated the predicted HbA_{1c} from fructosamine by initially converting the fructosamine value into its standard normal deviate (SND) and then the fructosamine SND was converted to HbA1c equivalents (F_HbA_{1c}) (3, 4):

SND[f] = (fructosamine − mean fructosamine)/SD fructosamine

F_HbA_{1c} = (SND[f] × SD HbA_{1c}) + mean HbA_{1c}.

GGap was thus calculated as the difference between the true HbA_{1c} and the fructosamine-derived standardized predicted F_HbA_{1c} (GGap = HbA_{1c} − F_HbA_{1c}). In this methodology the F_HbA_{1c} is not derived from HbA_{1c} by correlation/regression methods (3, 4). The normalized standard deviate reallocation of fructosamine levels yields fructosamine-based HbA_{1c} equivalent results with the same distribution, mean, and SD as HbA_{1c} without altering the rank position of fructosamine-derived values.

Recently, others have used glycated albumin rather than fructosamine to estimate GGap, although this has not been validated against the established method described above (31). Although fasting blood glucose estimations are correlated well with mean glucose, it is with a wide variance, and methodologies using 6- or 8-point glucose profiles provide better representation of mean glucose, and hence a relatively better metric to be used in the HGI calculation. Availability of continuous glucose monitoring (CGM) with high density of data and better reflection of postprandial peaks could help in mean glucose calculations [and most recently it has been suggested that CGM could be used to titrate HbA_{1c} and thus GGap for individual patients (2)], however, this has not yet been explored in HGI calculation. Nevertheless, note that mean glucose profiles were similar when comparing CGM and 8-point blood glucose testing (32).

Despite the availability of such new technologies in some arenas, it should be recognized that CGM is yet to be widely available in a large proportion of clinical situations and that HbA_{1c} and/or glycated albumin/fructosamine will continue to be the major methods of monitoring glycemia worldwide for many years. Crucially, the calculation of GGap and HGI will continue to provide important information in relationship to individual risk of diabetic complications as discussed below (see "Possible Contributions to GGap/HGI").

Alternative Explanations of GGap/HGI

The use of HbA_{1c} depends on the assumption that erythrocyte (intracellular) glucose concentrations are an accurate reflection of plasma (extracellular) glucose on the basis that erythrocytes express the constitutive glucose transporter GLUT1; however, this assumption may be incorrect for a number of reasons. Furthermore, the utility of glycated hemoglobin as an indicator of average glucose over the half-life of hemoglobin of 3 months assumes that there is no further change in the glycated product, and indeed that the half-life [or lifespan determined as the mean red blood cell age (M_{RBC})] of erythrocytes is consistent between individuals, which it demonstrably is not (2). Malka *et al.* (33) recently suggested a mathematical model for calculating individual M_{RBC}, which measure they propose as the explanation for all individual nonglycemic HbA_{1c} variability and as a correction to be used in patient-personalized assessment of HbA_{1c} results. GGap and HGI could potentially be explained

by genetic and racial differences in M_{RBC}, as pointed out by Cohen *et al.* (2), in addition to other non-glycemic factors, including alterations in GLUT1 expression or activity or intracellular enzymatic deglycation pathways, which are discussed below (see "Possible Contributions to GGap and HGI" and "Differential Rates of Intracellular Glycation Independent of Glucose: Fructosamine-3-Kinase as a Deglycating Enzyme Associated With GGap").

Consistency of GGap and HGI

It has been hypothesized that GGap and HGI represent a spurious statistical phenomenon (30, 34) arising from regression analysis used in some methodologies, and on this basis Lachin *et al.* (34) suggested that HGI is not completely glycemia-independent and hence not an independent predictor of complications (see "Association of Diabetes Complications With GGap and HGI" below). Our method of calculating GGap using the standard normal deviate (see "Definition and Calculation of GGap and HGI" above) avoids this problem (3, 4). Furthermore, the consistency of GGap and HGI mitigates against this criticism, and hence GGap and HGI have both been shown to be consistent in individuals over time, indicating a constant variation in intracellular glycation compared with extracellular glycation or glycemia as measured by serum fructosamine or MBG (4–6). In a retrospective study on 2263 individuals with diabetes, by using multiple simultaneously measured HbA_{1c} and fructosamine in the same individuals over an extended time period, we confirmed that GGap can be of substantial magnitude, that there is no significant within-subject variability in GGap, and that the direction of GGap is consistent despite significant changes in HbA_{1c} and fructosamine during the time period (4) (these findings are updated, extended, and explored in more detail below: see "Clinical Implications of GGap and HGI" and Figs. 1–3). Others have demonstrated such reproducibility of GGap; for example, Cohen *et al.* (5) reported the reproducibility of GGap in 65 paired HbA_{1c}/fructosamine estimations separated by 23 weeks in a population with diabetes. In a population not known to have diabetes, Yudkin *et al.* (35) and Gould *et al.* (36) showed that the discrepancy between HbA_{1c} relative to fasting and 2-hour blood glucose levels in an oral glucose tolerance test remained consistent during a 4.4-year period (29, 30). Similarly, consistency in HGI has been studied in 128 children with type 1 diabetes, and it was noted to be consistent during a 2-year study period (6). It was shown that individuals consistently had the same direction and magnitude of HGI from repeated measurements of HbA_{1c} and MBG during a 2-year period in a clinic population of children and adolescents with type 1 diabetes.

A comparison of GGap and HGI in 62 patients with type 1 diabetes confirmed that the two indices are highly correlated and consistent (37). In a study in monozygotic twins, GGap was suggested to be 69% inheritable, indicating the possibility of a genetic basis for GGap (38).

Recent studies examining GGap and HGI in Korean patients with type 2 diabetes, in which glycated albumin rather than fructosamine was used in the GGap calculation, have confirmed the correlation between these the two indices and also further demonstrated their consistency, and interestingly that patients with a high HGI/positive GGap had a higher incidence of insulin use, albeit in a small study group (39). Akatsuka *et al.* (40) suggested that the ratio of glycated albumin to HbA_{1c} in International Federation of Clinical Chemistry and Laboratory Medicine units is an accurate measure of GGap and might be useful as a reference for predicting risk of complications in children with type 1 diabetes.

Association of Diabetes Complications With GGap and HGI

GGap/HGI might alter an individual's risk of vascular complications for any given level of long-term glycemic control by modifying one of the key pathologic processes, namely, protein glycation and the formation of advanced glycation end products. Hypothesizing that GGap is a trivial nonsystematic event, unconnected to diabetes outcomes, it would not then be expected to be associated with distinct subpopulations of human diabetes or to have any sequelae in clinical outcomes. We have reported the direct associations between positive GGap and microvascular and macrovascular complications of diabetes that are logically consistent with the glycation mechanism for complications (41). Belonging to the consistently positive GGap group was significantly associated with worsening retinopathy (OR, 1.96; 95% CI, 1.31 to 2.9; $P = 0.001$), increasing urine albumin/creatinine ratio (OR, 1.85; 95% CI, 1.14 to 3.01; $P = 0.012$), and the presence of established macrovascular disease (OR, 1.91; 95% CI, 1.18 to 3.09; $P = 0.008$). Others have reported a similar relationship between GGap/HGI and retinopathy and nephropathy. Cohen *et al.* (5) suggested that GGap increased the risk of more advanced nephropathy 2.9-fold. Rodríguez-Segade *et al.* (42) examined 2314 patients with type 2 diabetes for a mean of 6.5 years, dividing the cohort into tertiles based on the average of all individual GGaps, and showed that the mean GGap predicts the progression of nephropathy. In a study analyzing the data from the Diabetes Control and Complications Trial (DCCT), HGI was shown to be a significant predictor of retinopathy and nephropathy (43). Furthermore, the HGI subgroup analysis of the Action to Control

"HbA_{1c} alone may not always be reliable for diagnostic purposes, with studies showing a low sensitivity of HbA_{1c} for diagnosis..."

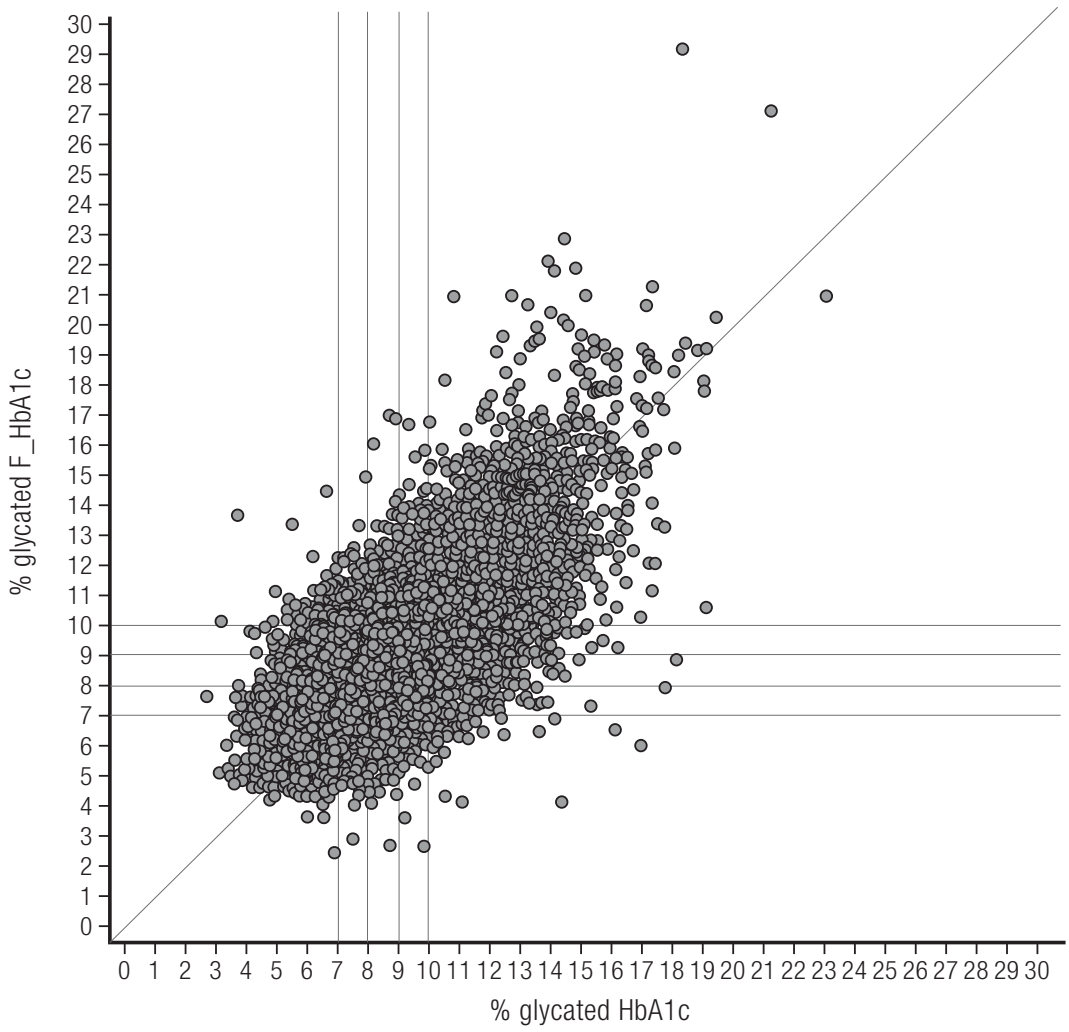

Figure 1. Clinical grid showing variation in categorization of actual HbA_{1c} and estimated fructosamine-derived HbA_{1c} in a single diabetes center during 10 years. Shown are 31,119 simultaneously measured HbA_{1c} and fructosamine estimations. F_HbA_{1c} is the derived HbA_{1c} estimated from fructosamine. The grid shows the levels at which HbA_{1c} and F_HbA_{1c} can be arbitrarily categorized as excellent [≤7% (53.0 mmol/mol)], good [7% to 8% (53.1 to 63.9 mmol/mol)], acceptable [8% to 9% (64.0 to 74.9 mmol/mol)], poor [9% to 10% (75.0 to 85.8 mmol/mol)], or very poor [>10% (85.8 mmol/mol)]. Glycated hemoglobin levels are depicted in DCCT units for simplicity. All values are shown with the degree of scatter around the line of unity, whereas horizontal and vertical lines represent the defined categories.

Cardiovascular Risk in Diabetes (ACCORD) trial revealed that intensive treatment significantly reduced the primary composite outcome (first occurrence of nonfatal myocardial infarction, nonfatal stroke, or death from other cardiovascular causes) by 25% in the low HGI subgroup and by 23% in the moderate HGI subgroup, but not in the high HGI subgroup, where the primary outcomes were similar between the standard and intensive glycemia treatment groups (44). In the ACCORD cohort, fasting glucose values were used to calculate the predicted HbA_{1c} and HGI, potentially not taking into consideration the effect of the postprandial glucose variations that may have impacted on HGI.

We also examined mortality in our cross-sectional, retrospective study and found that the adjusted all-cause mortality was higher (twofold) both in the negative and positive GGap groups compared with the neutral GGap cohort (41). Cid Alvarez *et al.* (45) in a prospective cohort study of individuals with diabetes and without diabetes, with acute coronary syndrome, demonstrated an association of increased mortality with higher GGap values [diabetes cohort hazard ratio (HR), 1.31; 95% CI, 1.14 to 1.50; $P < 0.001$; non-diabetic patients HR, 1.30; 95% CI, 1.04 to 1.64; $P = 0.018$). HGI subgroup analysis in the cohort from the ACCORD trial also suggested increased total mortality by 41% ($P = 0.02$) in the high HGI group but not in low and moderate HGI group, in those in the intensive treatment arm (44).

In the cohort of patients with type 2 diabetes studied in the Action in Diabetes and Vascular Disease trial, HGI was found to be a strong predictor of microvascular and macrovascular complications and mortality irrespective of the treatment allocation (intensive vs standard treatment) but no better than HbA_{1c}. Furthermore, it was noted that intensive control reduced mortality in the high HGI cohort (high measured HbA_{1c} relative to that predicted from MBG) (46). This result was inconsistent with the findings of the ACCORD trial; however, the difference in the treatment regimen in the two trials is likely to have contributed to the different findings.

Similar to our findings of the relationship of GGap with mortality (41), in a study of 976 individuals with diabetes with ischemic stroke, high and low HGI were linked to poor outcome, with a U-shaped association of HGI with prognosis being demonstrated (47).

Table 1 (5, 28, 34, 41, 44–52) summarizes the published studies demonstrating the association between GGap/HGI and diabetic complications.

Possible Contributions to GGap/HGI

Because GGap or HGI is a measure of the net difference between HbA_{1c} and fructosamine or MBG, factors that affect either of these parameters could influence GGap or HGI.

As previously indicated, the time frame of glycemic attainment represented by fructosamine is shorter than that of HbA_{1c}, the glycation it indicates may be influenced by protein turnover rates and protein loss as proteinuria, and these factors may play a part in GGap. Many have shown a good relationship between HbA_{1c} and fructosamine (3–5, 27). Fructosamine is known to be well associated with preceding blood glucose levels (19). A concern relating to a possibly confounding association of fructosamine levels with proteinuria has been raised; however, in our study utilizing regression analysis of fructosamine with multiple relevant clinical and biochemical factors, we showed that, overall, they explained ≤20% of the variance in fructosamine, which is to say 80% of fructosamine is not associated with any known influencing factor (41), among which the urine albumin/creatinine ratio had the statistically weakest independent association, with an r^2 of 0.002, thus representing only 0.2% of the accountable variance of fructosamine.

A variety of factors independent of prevailing glycemia influence glycated HbA_{1c}. Genetic variations could influence HbA_{1c} through nonglycemic pathways and contribute to HbA_{1c}/glycemia discordance (53). A previous study has confirmed that GGap may be partly genetically determined and account for one third of the heritability of HbA_{1c} (38).

Bergenstal *et al.* (54) demonstrated a racial difference in the relationship between HbA_{1c} and glycemia, confirming that HbA_{1c} levels overestimate the mean glucose concentration in blacks compared with whites, suggesting that there may be racial differences in the glycation of hemoglobin. Such ethnic differences that have a likely genetic component have also been demonstrated in a new study by Hivert *et al.* (55), and the authors point out that an association of a genetic variant in *G6PD*, which is common in blacks, is a factor identified in genome-wide association studies as a genetic determinant of Hb_{A1c} (56). The importance of the effect of HbS found mainly in African American populations on Hb_{A1c} assays is also apparent (57), although this is an effect of which laboratories are aware and therefore normally allow for. Factors that impact red cell survival or those that regulate intracellular glucose concentrations—including glucose permeability across the red cell membrane, independent of extracellular glucose—have been shown also to contribute to the extent of hemoglobin glycation (23–25). The variability in intracellular glucose relative to extracellular glycemia significantly contributes to variation in HbA_{1c}. Other factors that influence nonenzymatic hemoglobin glycation include intracellular pH, 2,3-diphosphoglycerate concentration, and glycolytic enzyme activity (36).

Differential Rates of Intracellular Glycation Independent of Glucose: Fructosamine-3-Kinase as a Deglycating Enzyme Associated With GGap

One possible explanation of GGap that has been mooted is that of an enzyme-mediated intracellular deglycation process. We have recently adduced evidence of a potential role of the enzyme fructosamine-3-kinase (FN3K) enzyme in GGap. FN3K has previously been shown to phosphorylate (aldimine) Amadori products of protein glycation at specific amino groups in hemoglobin and other proteins, effectively deglycating the protein and restoring the free amino group with the production of deoxyglucosone (58). FN3K is a predominantly intracellular enzyme

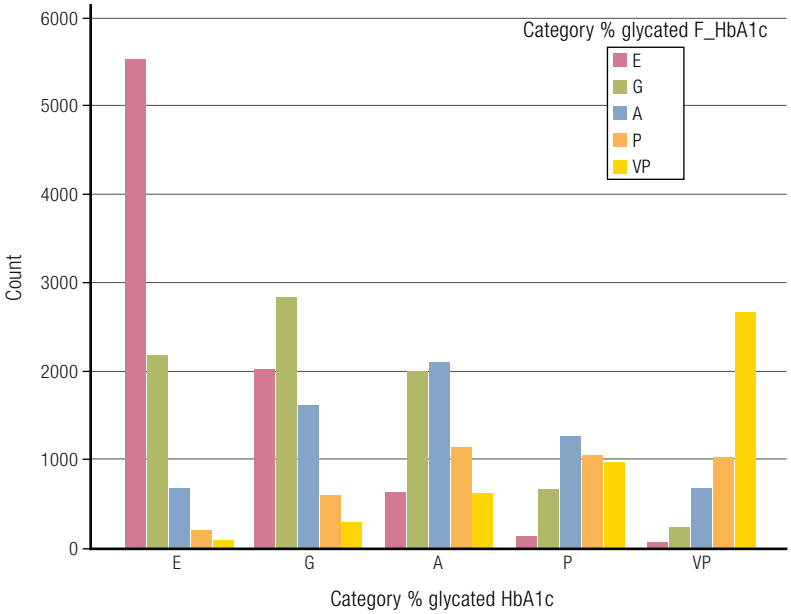

Figure 2. Differences in clinical categorization using actual HbA_{1c} and estimated fructosamine-derived HbA_{1c} (F_HbA_{1c}). Categories of HbA_{1c} and F_HbA_{1c} based on the data depicted in Fig. 1 are compared. The glycemic control categories on the *x*-axis (E, excellent; G, good; A, acceptable; P, poor; VP, very poor) are those defined using actual HbA_{1c}, and the colors show the categories that would be derived for the same measurements if based on F_HbA_{1c}. Red indicates excellent; green, good; blue, acceptable; orange, poor; and yellow, very poor.

expressed highly in erythrocytes (59). Single-nucleotide polymorphisms in the FN3K gene have been shown to be associated variously with HbA_{1c} levels, and circulating soluble receptors for advanced glycation end product (AGE) (60, 61) and genome-wide association studies have found the *fn3k* gene to be one of the top hits for association with HbA_{1c} (62, 63), with this genetic variant not being associated with glycemic traits or erythrocytic indices.

We studied erythrocyte FN3K concentrations and enzyme activity in a subset of our diabetes patient population, dichotomized for a large positive or negative GGap (64). We showed that FN3K protein was significantly higher and, strikingly, that FN3K enzyme activity was threefold greater at any given FN3K protein level in the erythrocytes of the negative GGap groups compared with positive GGap groups. This was associated with significantly lower AGE levels, lower proinflammatory adipokines (leptin/adiponectin ratio), and much lower prothrombotic PAI-1 levels in the negative GGap cohort, thus suggesting a possible role of FN3K as a deglycating enzyme in diabetes complications, potentially reducing some of the AGE involved in the pathogenesis of diabetes complications (64).

An objection to the potential role of FN3K in GGap arises from its higher rate of activity in respect of fructosyl-lysines generated by glycation of side-chain amino groups in proteins, coupled to its reportedly low activity on N-terminal amino groups such as that of the N-terminal valine on hemoglobin β-chains (65). Thus, the specificity of FN3K to *N*-ε-fructosyl-lysine (FruLys) compared with N-terminal *N*-α-fructosyl amino acids reportedly ranges from 100- to 10-fold lower affinity (66). This argument may be countered by considering the long time period during which FN3K may be able to act within the erythrocyte, and because a lower affinity simply suggests a slower, but not zero-rate, reaction (especially if the difference is only 10-fold), a significant degree of deglycation at the N-terminal valine may still occur. It must also be considered that published affinity values for FruLys comprise the free amino acid and the protein-bound or histone-bound FruLys, whereas for *N*-α–bound Amadori products, only the free amino acids have been examined (58, 59). Indeed, there is preliminary evidence of significantly measurable activity on *N*-α–bound Amadori products such as fructosyl-valine (67). That our studies have demonstrated such a marked difference in FN3K activity in relationship to GGap also supports the contention that it has a significant role in GGap (64).

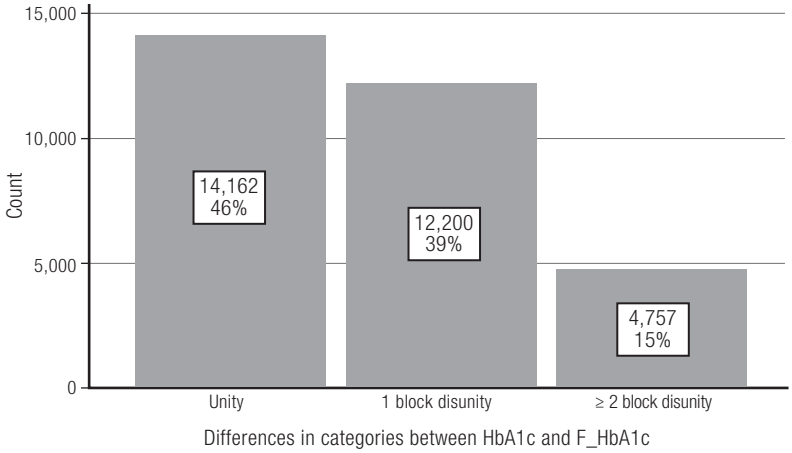

Figure 3. Shown is the magnitude of the variance between the glycemic categories defined in Fig. 2. This sums all variations that are in agreement, those that are 1 block of category different, or those that are ≥2 blocks of category different. Fewer than half (46%) of the paired measurements give categorization results that agree when comparing HbA_{1c} and F_HbA_{1c}; 54% disagree by at least 1 block of category and, of these, 15% disagree by 2 blocks of category or more, potentially leading to serious clinical misjudgments.

Clinical Implications of GGap and HGI

The disagreement between HbA_{1c} and other measures of glycemia, including fructosamine or MBG, as calculated by GGap or HGI, respectively, can be substantial in magnitude and is consistent over time (3–6, 27). Thus, utilizing HbA_{1c}, the current gold standard for assessment of glycemic control in diabetes, alone could potentially underestimate or overestimate the prevailing glycemia, leading to error in clinical assessment and management. Moreover, use of the derived estimated average glucose is more likely to result in overlooking the limitations of the HbA_{1c} measurement from which it is calculated (68). Individuals with a high GGap or high HGI, wherein the HbA_{1c} is higher than indicated by the serum fructosamine or MBG, respectively, may receive an uptitration of their glycemia treatment that may put them at undue risk of hypoglycemia when GGap/HGI are not taken into account (and several studies confirm that this does happen in practice). Alternatively, in the case of those with a negative GGap/low HGI, wherein the HbA_{1c} is lower than the prevailing glycemia, clinicians may be falsely reassured by HbA_{1c}, resulting in no appropriate therapy intensification to improve glycemia, putting such individuals at risk for diabetes-related complications. The HGI subgroup analysis in the ACCORD trial suggested that the incidence of hypoglycemia was progressively higher in the low, moderate, and high HGI subgroups in both intensive (14.5%, 16.8%, and 18.8%, respectively) and standard (3.7%, 4.5%, and 7.5%, respectively) glycemia treatment cohorts (44).

HbA_{1c} arguably still has value as a risk marker in diabetes risk stratification, prediction, diagnosis, and management for many patients where clinical errors may be small; however, the danger of not questioning its validity in the subset of individuals where there is potential for large errors is apparent from the

Table 1. Studies Investigating the Association of Diabetic Complications With GGap and HGI

Study (Ref.)	Patient Population	Results
GGap and complications		
Cohen *et al.*, 2003 (5)	40 patients with type 1 diabetes of >15 y duration	GGap increase by 1% was associated with 2.9-fold greater frequency of adverse nephropathy stage ($P = 0.0014$)
Rodríguez-Segade *et al.*, 2011 (42)	2314 patients with type 2 diabetes	High GGap associated with progression of nephropathy in type 2 diabetes
		HR (high vs low GGap), 2.52 ($P < 0.001$) and HR (medium vs low GGap), 1.61 ($P = 0.001$)
Nayak *et al.*, 2013 (41)	3182 patients with type 1 and type 2 diabetes	Positive GGap is associated with retinopathy (OR, 1.24; 95% CI, 1.01 to 1.52; $P = 0.039$), nephropathy (OR, 1.55; 95% CI, 1.23 to 1.95; $P = 0.008$), and macrovascular disease (OR, 1.91; 95% CI, 1.18 to 3.09; $P = 0.008$)
		GGap had a U-shaped quadratic relationship with mortality: negative G-gap (OR, 1.96; 95% CI, 1.50 to 2.55; $P < 0.001$) and positive G-gap (OR, 2.02; 95% CI, 1.57 to 2.60; $P < 0.001$) being associated with a significantly higher mortality.
Cosson *et al.*, 2013 (48)	925 patients with type 2 diabetes	High GGap (third tertile of GGap) was associated with macroproteinuria (OR, 1.6; 95% CI, 1.2 to 2.1; $P < 0.01$) independent of HbA_{1c}
Cid Alvarez *et al.*, 2012 (45)	1137 patients admitted with acute coronary syndrome	GGap was associated with a significantly higher risk of all-cause mortality in both patients with diabetes (HR, 1.31; 95% CI, 1.14 to 1.50; $P = 0.000$) and nondiabetic patients (HR, 1.30; 95% CI, 1.04 to 1.64; $P = 0.018$).
HGI and complications		
McCarter *et al.*, 2004 (41)	1441 DCCT participants with type 1 diabetes	High HGI group had greater risk of retinopathy (threefold) and nephropathy (sixfold) compared with low HGI group
Lachin *et al.*, 2007 (34)	1441 DCCT participants with type 1 diabetes	The effect of HGI on microvascular complications in DCCT cohort is wholly explained by the associated level of HbA_{1c}
Hempe *et al.*, 2015 (44)	10,251 patients with type 2 diabetes (ACCORD cohort)	Total mortality in intensively treated patients was higher in high HGI subgroup (HR, 1.41; 95% CI, 1.10 to 1.80)
		High HGI was associated with a greater risk for hypoglycemia in the standard and intensive treatment groups
van Steen *et al.*, 2018 (46)	11,083 patients with type 2 diabetes (Action in Diabetes and Vascular Disease trial cohort)	High HGI is a predictor of microvascular and macrovascular complications and mortality but no better than HbA_{1c}
		High HGI associated with lower risk for mortality when on intensive treatment
Rhee *et al.*, 2017 (49)	2052 nondiabetic individuals	High HGI associated with higher risk for incident coronary artery calcifications independent of HbA_{1c}
Fiorentino *et al.*, 2017 (50)	1120 whites without diabetes	High HGI associated with twofold increased risk of hepatic steatosis in nondiabetics
Cheng *et al.*, 2017 (51)	423 individuals with type 2 diabetes (Taiwan)	HGI correlated with the extent of coronary heart disease in individuals with type 2 diabetes
Marini *et al.*, 2017 (52)	2055 white nondiabetic adults age ≥18 y	HGI is a predictor of carotid intima–media thickness; individuals with high HGI had a 2.7-fold increased risk of vascular atherosclerosis (OR, 2.72; 95% CI, 1.01 to 7.37) as compared with individuals with low HGI
Pan *et al.*, 2017 (47)	976 diabetic patients with ischemic stroke (China)	Both high and low HGI were linked to poor outcome in acute ischemic stroke [U-shaped association with OR (95% CI) for low vs moderate HGI group = 1.64 (1.13 to 2.38), $P = 0.01$, and high vs moderate HGI = 1.54 (1.06 to 2.24), $P = 0.02$]
Ahn *et al.*, 2017 (28)	248 treatment-naive subjects with prediabetes or diabetes	Highest HGI tertile was independently associated with composite cardiovascular disease (OR, 2.81; 95% CI, 1.59 to 4.98), individual cardiovascular disease (OR, 2.30; 95% CI, 1.12 to 4.73), stroke (OR, 3.40; 95% CI, 1.50 to 7.73), and peripheral artery disease (OR, 6.37; 95% CI, 1.18 to 34.33) after adjustment for other cardiovascular disease risk factors, including HbA_{1c} levels

discussion in the preceding paragraph. Setting aside considerations of its calculation, its impact on vascular risk and mortality, and its possible underlying etiology, the key purpose of this review is to raise awareness of the potential of HbA_{1c} inaccuracy for this subset of patients, reflected in GGap (and HGI), to result in significant error in the assessment of glycemic control. To assess the possible scale of this error (and thus the probable size of the patient subset), we extended and reanalyzed the data previously published (3) and we have recently reported the findings of this reanalysis (69). These findings are highlighted in Fig. 1, which shows our total accrued data on 31,119 simultaneously measured HbA_{1c} and fructosamine estimations undertaken in our single center during 10 years. It is apparent from this figure that there is wide scatter around the line of unity which, in absolute terms, was >1U of glycated HbA_{1c}% or 7.2 mmol/mol in 40% of estimations.

Figure 2 shows how this scatter results in differences in the categorization of glycemic attainment between HbA_{1c} and F_HbA_{1c}. Although many people with diabetes in our center would be accurately categorized for glycemic attainment based on HbA_{1c}, a large proportion would not. This is demonstrated in Fig. 3, which depicts the magnitude of the variance in that categorization, with only 46% showing concordance and 15% of patients having, in our opinion, a large enough difference to impart certain risk by way of error in a clinician's judgment, with consequent potential for inappropriate therapy and management.

It may thereby be, as reported by ACCORD (42), that those in lower attained HbA_{1c} brackets with a higher HGI, who were perhaps exposed to therapy intensification inappropriately, came to harm. A recent review by Campbell *et al.* (24) suggested that GGap is unlikely to cause such errors because "in the main initiation and alterations of diabetic therapies are almost never made based on an isolated HbA_{1c}, particularly at levels close to the diagnostic threshold"; however, as we have seen, because of the individually consistent nature of GGap, multiple measurements of HbA_{1c} are likely to yield similar conclusions, and furthermore, as we have shown in the preceding paragraph, common variations as small as 1% in HbA_{1c} from mean glycemia-predicted HbA_{1c} can result in significant clinical errors. This is further supported by Cohen *et al.* (2), quoting a recent study by Rhee *et al.* (70), who found in a "VA population that those whose HbA1c is highest relative to blood glucose [*i.e.*, equivalent to a positive GGap] had a 56% higher frequency of ER visits for hypoglycemia than those whose HbA1c is either proportionate or lowest for blood glucose [*i.e.*, a neutral or negative GGap]" (insertions in brackets are ours).

Understanding the association of GGap and HGI with key diabetes-related microvascular complications, as suggested in various studies, in a pattern consistent with a key pathophysiological mechanism, namely glycation of proteins, would help to risk-stratify such individuals for targeted risk reduction therapies, and also help in the future to develop pharmacological interventions aimed toward risk reduction. The observations of significantly different outcomes in the different HGI subgroups in the ACCORD trial in response to intensive treatment strongly supports the need for more personalized diabetes management and suggests that HGI could be used to help individualize treatment goals.

Given the standardization of HbA_{1c} assays, ease of its estimation, and practicality of its use in treatment modification (based on available evidence for association with complications and improved outcomes with HbA_{1c} reduction), HbA_{1c} is now adopted internationally for the diagnosis of diabetes. However, the magnitude of GGap/HGI means that this reliance may have marked impact in a significant minority of cases. HbA_{1c} alone may not always be reliable for diagnostic purposes, with studies showing a low sensitivity of HbA_{1c} for diagnosis, leading to a substantial number of missed diagnoses and to error in classification of diabetes status, in the absence of concurrent use of available glucose criteria for diabetes diagnosis. Rodríguez-Segade *et al.* (71), in a study on patients with previously undiagnosed diabetes, confirmed that the differences between HbA_{1c}-based and fasting plasma glucose/oral glucose tolerance test–based diagnoses are largely due to the influence of GGap calculated using simultaneously measured serum fructosamine. As previously mentioned, the increasing availability of CGM in some areas has the potential to provide an alternative to HbA_{1c} in assessing glycemic control, and Beck *et al.* (72) and Bergenstal *et al.* (73) have suggested the use of a factor derived from CGM that they term the glucose management indicator (72, 73). However, as pointed out earlier (see the end of "Definition and Calculation of GGap and HGI" above), CGM is still not available in many constituencies and in others is limited (for funding and other reasons) to a small fraction of diabetes patients (*e.g.*, in the United Kingdom, where strict National Health Service guidelines restrict provision to patients with type 1 diabetes having poor control, *i.e.*, substantially <5% of patients with diabetes); furthermore, as we describe in the previous paragraph, determination of GGap or HGI (unlike the glucose management indicator) has the additional benefit of providing a prognostic indicator of risk of diabetic complications.

Conclusions

In summary, GGap/HGI can be sufficient in magnitude to cause an error in the judgment of glycemia attainment.

Hence, the incorporation of GGap/HGI during assessment of glycemic control would help to ascertain how far HbA_{1c} diverges from alternative estimates of glycemia to avoid misinterpretation of glycemic control and to avoid inappropriate therapeutic management. Understanding the consistency of GGap, its association with a phenotype in diabetes, microvascular complications, macrovascular disease, and possibly mortality, and its possible mechanistic association with FN3K enzyme activity will also contribute toward directing further research into emerging therapeutic interventions to lessen diabetes-related complications.

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Abbreviations

ACCORD, Action to Control Cardiovascular Risk in Diabetes; AGE, advanced glycation end product; CGM, continuous glucose monitoring; DCCT, Diabetes Control and Complications Trial; F_HbA_{1c}, fructosamine-derived HbA_{1c}; FN3K, fructosamine-3-kinase; FruLys, *N*-ε-fructosyl-lysine; GGap, glycation gap; HGI, hemoglobin glycation index; HR, hazard ratio; MBG, mean blood glucose; M_{RBC}, mean red blood cell age..