Association of Glycation Gap With Mortality and Vascular Complications in Diabetes

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OBJECTIVE—The “glycation gap” (G-gap), an essentially unproved concept, is an empiric measure of disagreement between Hba1c and fructosamine, the two indirect estimates of glycemic control. Its association with demographic features and key clinical outcomes in individuals with diabetes is uncertain.

RESEARCH DESIGN AND METHODS—The G-gap was calculated as the difference between measured Hba1c and a fructosamine-derived standardized predicted Hba1c in 3,182 individuals with diabetes. The G-gap’s associations with demographics and clinical outcomes (retinopathy, nephropathy, macrovascular disease, and mortality) were determined.

RESULTS—Demographics varied significantly with G-gap for age, sex, ethnic status, smoking status, type and duration of diabetes, insulin use, and obesity. A positive G-gap was associated with retinopathy (odds ratio 1.24 [95% CI 1.01–1.52], P = 0.039), nephropathy (1.55 [1.23–1.95], P < 0.001), and, in a subset, macrovascular disease (1.91 [1.18–3.09], P = 0.008). In Cox regression analysis, the G-gap had a “U”-shaped quadratic relationship with mortality, with both negative G-gap (1.96 [1.50–2.55], P < 0.001) and positive G-gap (2.02 [1.57–2.60], P < 0.001) being associated with a significantly higher mortality.

CONCLUSIONS—We confirm published associations of G-gap with retinopathy and nephropathy. We newly demonstrate a relationship with macrovascular and mortality outcomes and potential links to distinct subpopulations of diabetes.

T he glycation gap (G-gap) refers to the potential deviation of glycated Hba1c away from the other indirect estimate of blood glucose attainment such that it might read substantially lower or higher than expected (1–3). Glycated Hba1c represents the net effect of several mechanisms, which may shift its direct glycation relationship with overall levels of glycemia (4–6). Many factors are known to influence Hba1c, including various erythrocytic processes (6–9). Protein glycation is a nonenzymatic reaction dependent on glucose concentrations, but intracellular enzymatic deglycation of proteins has also been identified (10). The key deglycating enzyme, fructosamine-3-kinase, has isoforms and a genetic polymorphism suggested to influence Hba1c variability, but any impact on Hba1c glycation is unknown; although it seems unlikely that glycated Hba1c is a substrate for this enzyme since it has been shown that there is no evidence that it plays any role in Hba1c deglycation at the relevant glycation site (11,12). To add to the potential for a spurious generation of a G-gap, many factors, including variability in protein turnover and obesity, may affect fructosamine estimation (1,13,14). The evidence concerning the effects of urinary protein loss are mixed (1,13). Even then, fructosamine reflects blood glucose attainment over a much shorter time frame than Hba1c and may more readily be influenced by very short-term changes in blood glucose levels. It may simply be that the G-gap is no more than an empiric and potentially spurious measure of disagreement between the two indirect estimates of glycemic control, with each having a number of confounders to the direct relationship with blood glucose.

Although we have demonstrated that the G-gap is a consistent phenomenon within individuals over time (1), there remains doubt as to whether the G-gap is a real phenomenon or if it has any significant sequelae (15). Hypothesizing that the G-gap is an inconsequential nonsystematic event, irrelevant 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. This paper explores the association of the G-gap with diabetic population demographic factors and with crucial clinical outcomes to determine if such associations exist.

RESEARCH DESIGN AND METHODS

Patient selection
We reviewed all Hba1c and fructosamine estimations undertaken at New Cross Hospital over 4 years (2006–2009), identifying and selecting all adults with diabetes (≥18 years of age) who had paired estimations of Hba1c and fructosamine performed on the same day from the same sample set. Thereafter, clinical information was taken from our diabetes registry and linked to this dataset. The diabetes register is validated to be >99% accurate for the identification of known diabetes and for mortality status in linkage with the National Health Service Strategic Tracing Service. Pregnant women, those with a creatinine >200 μmol/L, those with a known hemoglobinopathy, or those with an abnormal electrophoretic pattern on Hba1c testing were excluded.

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Received 30 May 2012 and accepted 29 April 2013.
DOI: 10.2337/dc12-1040
This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc12-1040/-/DC1.
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Glycation gap and mortality in diabetes

Retinopathy grading, microalbuminuria, and macrovascular risk

Digital retinal screening was in accordance with the National Screening Program for Diabetic Retinopathy (ENSPRD) (16). Retinopathy was categorized into a dichotomized variable (with or without any retinopathy). Urine albumin-creatinine ratio (UACR) was assessed as dichotomous variable dividing into lower risk or higher risk for progressive microalbuminuria (<10 or >10 mg/mmol) (17). Individuals were categorized as having established macrovascular disease depending on the presence or absence of any previous cardiac, cerebral, or peripheral macrovascular event.

Analytical methods

HbA1c. International Federation of Clinical Chemistry values were available only from 1 June 2009. Hence we have used the Diabetes Control and Complications Trial (DCCT)-aligned HbA1c in our analysis. HbA1c was measured using high-performance liquid chromatography on a Tosoh G7 analyzer (Tosoh Bioscience Ltd., Worcestershire, U.K.). The performance scores in the UK National External Quality Assurance Scheme (UK NEQAS) for glycated hemoglobins (maximum limits: A (accuracy) score <100 and B (bias) score <2%, which were within the acceptable limits of the UK NEQAS for glycated hemoglobins (maximum limits: A score <200 and B score less than ±7.5%). The between-batch coefficient of variation was 1.8 and 1.4% for an HbA1c of 5.7% (39 mmol/mol) and 9.5% (80 mmol/mol), respectively. Fructosamine was measured by nitro tetrazolium-blue reduction on a Roche Modular P analyzer (Roche Diagnostics Ltd., West Sussex, U.K.) using a Cobsit with between-batch coefficient of variation 3.1% at a level of 263 µmol/L and 2.2% at 518 µmol/L (18).

Calculation of the fructosamine-predicted HbA1c and the G-gap

As published (1), a predicted HbA1c (FHbA1c) was calculated from the simultaneously measured fructosamine standardized to the HbA1c distribution according to the following equation: FHbA1c = [(fructosamine – mean fructosamine)/SD fructosamine] × SD HbA1c + mean HbA1c. The G-gap was the difference between the true HbA1c and the fructosamine-derived standardized predicted FHbA1c (G-gap = HbA1c – FHbA1c). Importantly the FHbA1c was not derived from HbA1c by correlation/regression methods. The normalized standard deviate reallocation of fructosamine levels yields fructosamine-based HbA1c equivalent results with the same distribution, mean, and SD as HbA1c without altering the rank position of the fructosamine-derived value. A negative G-gap denotes the true HbA1c appearing to read lower than the FHbA1c, and a positive G-gap denotes the true HbA1c appearing to read higher than that predicted by fructosamine. Among those with a second paired HbA1c-fructosamine estimation, in order to identify those with a consistent G-gap direction, the product of two G-gaps was calculated. If consistent, the G-gap product would be positive (positive × positive = positive; negative × negative = positive), but any discordance in direction of the G-gap over time in two paired readings would yield a negative G-gap product (negative × positive = negative).

For the whole cohort (n = 3,182), HbA1c was associated significantly (r² = 0.10, F = 26.9, P < 0.001) with age, sex, diabetes duration, smoking status, retinopathy status, serum albumin, and UACR but not with ethnicity, diabetes type, BMI, serum creatinine, estimated glomerular filtration rate (eGFR), macrovascular disease, or mortality status. Fructosamine (r² = 0.20, F = 62.4, P < 0.001) was associated with age, ethnicity, diabetes type, diabetes duration, BMI, retinopathy grade, and UACR but not with sex, smoking status, serum creatinine, eGFR, serum albumin, macrovascular disease, or mortality status. The actual versus regression model-predicted values (incorporating the statistical effect of the factors outlined above) for HbA1c (8.6 ± 1.8 vs. 8.6 ± 0.6%) and fructosamine (313 ± 74 vs. 313 ± 33 µmol/L) were not significantly different and the crude values were thus used throughout.

G-gap categorization

The G-gap (unit = HbA1c %) was categorized as negative, neutral, or positive when less than or equal to −1 (i.e., more negative than −1), greater than −1 to less than +1, or greater than or equal to +1, respectively. This categorization was taken from our previously published clinical error grid analysis of the impact of G-gap on assessment of glycemic control (19).

Statistical analysis

Data were analyzed on SPSS version 19. Comparison between multiple group means was by one-way ANOVA and the differences between frequencies/proportions by χ² test. Binary logistic regression was used to determine the association of various independent factors with dichotomized variables. Survival analysis was undertaken using Cox regression. In each case, a stepwise backward extraction method excluded all nonsignificant variables (P > 0.05) to determine the simplest, most parsimonious model. Data are presented as the mean ± SD. All statistical tests were considered significant at P < 0.05.

Ethical committee approval

The use of the clinical database for this study was approved by the relevant local U.K. National Health Service Research Ethical Committee.

RESULTS—Of 4,757 patients identified, 3,182 had complete demographic data and were included. Their follow-up from the first paired HbA1c-fructosamine estimation to the time of death or study end point was 38 ± 16 months.

Table 1 shows the glycation estimates. The correlation between HbA1c and fructosamine in the first HbA1c-fructosamine pair is r = 0.75, P < 0.001 (n = 3,182). Nevertheless, the G-gap range demonstrates the substantial magnitude of variation between HbA1c and FHbA1c, both of which indicated completely differing assessments of attainment of glycemic control. The distribution of G-gap status for the whole group varied significantly by HbA1c quintile (χ² = 505.6, P < 0.001), noting the striking increase in negative G-gap status in the lowest HbA1c quintile, whereas the positive G-gap prevalence was graded across ascending quintiles (Fig. 1). Repeat HbA1c-fructosamine estimations were undertaken 11 ± 10 months after the first in 1,609 patients. There was a quadratic relationship (r² = 0.67, P < 0.001) between the first and second G-gap (as described in RESEARCH DESIGN AND METHODS) with only 47 (3%) and 17 (1%) of the 1,609 patients discordant at a G-gap product more negative than −0.5 and −1.0, respectively.

There were significant differences between G-gap categories in a number of relevant demographic characteristics (Table 1). The key clinical outcomes of retinopathy (borderline significance), nephropathy (UACR), established macrovascular disease, and mortality also varied significantly with G-gap status (Table 1).
Binary logistic regression analyses were undertaken to determine the relationship between the absence or presence of these diabetes outcomes and the G-gap categories (negative, neutral, and positive, as defined), taking into account other identified relevant significant factors (age, sex, ethnic status, smoking status, diabetes type, duration of diabetes, insulin use, and BMI). The overall models were all significant \((P < 0.001)\) for each outcome (Table 2). Within that, independent of the other significant factors, the G-gap effect was significant for retinopathy and UACR, and the outcomes were worse with a positive G-gap category (Table 2). The G-gap status did not retain significance with macrovascular disease prevalence \((P = 0.28)\) after regression model adjustment for other factors.

When considering only those 1,609 with repeat estimates and consistency in their G-gap over time and repeated measures, 549 had a consistently negative (less than or equal to \(-1\) HbA\(_1c\) \([n = 235]\) or positive (greater than or equal to \(+1\) HbA\(_1c\) \([n = 314]\) G-gap. Belonging to the consistently positive G-gap group was significantly associated with worsening retinopathy (odds ratio [OR] 1.96 [95% CI 1.31–2.9], \(P < 0.001\)), increasing UACR (1.85 [1.14–3.01], \(P = 0.012\)) but now also the presence of established macrovascular disease (1.91 [1.18–3.09], \(P = 0.008\)).

The mortality pattern with G-gap differed and was clearly not linear but rather “U” shaped. In Cox regression analysis, the G-gap association was significant only as a quadratic nonlinear U-shaped relationship (overall \(\chi^2 = 307.3, P < 0.001\)). The significant factors were age \((P < 0.001)\), smoking \((P < 0.001)\), ethnicity \((P = 0.003)\), and G-gap (squared term) \((P < 0.001)\) but not sex, BMI, type or duration of diabetes, and insulin use. Introducing the prevailing HbA\(_1c\) (latest value) into the model had no significant effect \((P = 0.082)\).

Similarly the G-gap retained its significant association with mortality independent of proteinuria. Indeed proteinuria lost its significant association with mortality when the most heavily proteinuric subjects were excluded (UACR >200 mg/mmol) whereas G-gap retained significance. Furthermore, G-gap continued to be significantly associated with mortality even when a completely normoalbuminuric population subset was analyzed \((n = 2,077; 1,873 alive and 204 dead; \chi^2 = 179.02, P < 0.001; \) G-gap OR 1.05 [1.02–1.09], \(P < 0.001)\). See Supplementary Data online for Supplementary Table 1 and annotation.

Table 1—The biochemical, demographic, and clinical characteristics of the cohort, categorized according to G-gap status

<table>
<thead>
<tr>
<th>G-gap category (HbA(_1c) %)</th>
<th>Negative ((\leq -1))</th>
<th>Neutral ((&gt;-1) to (&lt;+1))</th>
<th>Positive ((\geq +1))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%)</td>
<td>586 (18)</td>
<td>1,945 (61)</td>
<td>651 (21)</td>
</tr>
<tr>
<td>HbA(_1c) (%)</td>
<td>7.9 ± 1.8</td>
<td>8.2 ± 1.5</td>
<td>9.9 ± 2.0</td>
</tr>
<tr>
<td>Fructosamine ((\mu)mol/L)</td>
<td>365 ± 91</td>
<td>301 ± 66</td>
<td>298 ± 75</td>
</tr>
<tr>
<td>FHbA(_1c) (%)</td>
<td>9.7 ± 2.1</td>
<td>8.2 ± 1.5</td>
<td>8.2 ± 1.7</td>
</tr>
<tr>
<td>G-gap (HbA(_1c) %)</td>
<td>(-1.8 ± 0.8)</td>
<td>0.0 ± 0.5</td>
<td>+1.7 ± 0.8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61 ± 18</td>
<td>66 ± 14</td>
<td>64 ± 13</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>63</td>
<td>54</td>
<td>47</td>
</tr>
<tr>
<td>Ethnicity (% white, Asian, black)</td>
<td>68, 6, 16</td>
<td>62, 28, 10</td>
<td>62, 32, 6</td>
</tr>
<tr>
<td>Smoking status (% never, ex, current)</td>
<td>64, 27, 9</td>
<td>62, 29, 9</td>
<td>57, 28, 15</td>
</tr>
<tr>
<td>Diabetes type (% type 2)</td>
<td>60</td>
<td>85</td>
<td>90</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>20 ± 11</td>
<td>17 ± 9</td>
<td>16 ± 8</td>
</tr>
<tr>
<td>On insulin (%)</td>
<td>76</td>
<td>68</td>
<td>76</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>28.8 ± 5.6</td>
<td>31.8 ± 6.1</td>
<td>34.4 ± 7.4</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>136 ± 21</td>
<td>138 ± 21</td>
<td>137 ± 12</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>73 ± 12</td>
<td>73 ± 12</td>
<td>74 ± 11</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.4 ± 1.1</td>
<td>4.3 ± 1.0</td>
<td>4.4 ± 1.2</td>
</tr>
<tr>
<td>Retinopathy (% any)</td>
<td>62</td>
<td>61</td>
<td>66</td>
</tr>
<tr>
<td>UACR (% &gt;10 mg/mmol)</td>
<td>15</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>UACR (mg/mmol)</td>
<td>10 ± 56</td>
<td>12 ± 48</td>
<td>22 ± 70</td>
</tr>
<tr>
<td>Serum creatinine ((\mu)mol/L)</td>
<td>93 ± 30</td>
<td>92 ± 29</td>
<td>87 ± 28</td>
</tr>
<tr>
<td>eGFR (ml/min)</td>
<td>80 ± 27</td>
<td>76 ± 26</td>
<td>79 ± 27</td>
</tr>
<tr>
<td>Macrovascular risk (% established)</td>
<td>24</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>Vital status (number [%] dead)</td>
<td>87 (15)</td>
<td>196 (10)</td>
<td>108 (16)</td>
</tr>
</tbody>
</table>

CONCLUSIONS—The entire notion of a G-gap must be treated with skepticism and caution. There are extensive confounding factors that are real caveats to its meaning. Whether they relate to erythrocytic function, biochemical pathways, or pure statistical error, mechanisms for the G-gap are, as yet, not at all understood. It would be appropriate to clearly state that there is no known genetic or biochemical mechanism that provides anything remotely close to a definitive explanation.

Yet, we now show that the G-gap varies significantly with demographic characteristics and is associated with the key diabetes outcomes: retinopathy, nephropathy, macrovascular disease, and mortality. Variation in demographics with the G-gap status has not been previously reported. The G-gap is consistent...
Glycation gap and mortality in diabetes

![Figure 1](Image)

**Figure 1**—The prevalence of negative and positive G-gap status by HbA1c quintile ($\chi^2 = 505.8$, P < 0.001). The HbA1c (mean [range]) for the quintiles of HbA1c: 1) 6.4% (3.8–7.0), 2) 7.5% (7.1–7.8), 3) 8.3% (7.9–8.6), 4) 9.2% (8.7–9.7), and 5) 11.3% (9.8–19.0).

over time (1–3), and twin studies suggest it to have significant inheritability (20). G-gap consistency, potential inheritability, and the now-reported demographic linkages tantalizingly point toward human diabetic subpopulations with biological variation in any underlying pathophysiological mechanisms.

Hyperglycemia is central to the development of diabetes complications (21–23). Hyperglycemia-induced protein glycation is an unequivocally important pathophysiological mechanism (21,24). Any factors significantly altering glycation may theoretically alter the relationship between glucose and the development of diabetes complications. Glycated HbA1c has been shown to correlate with the risk for developing microvascular complications in diabetes (22,23). The G-gap is proposed as a measure of the deviation of glycated HbA1c away from its expected value, such that a negative G-gap is taken as meaning a lesser level of glycation than expected and a positive G-gap more so. Our observations, that the micro- and macrovascular complications of diabetes are directly associated with a positive G-gap, are logically consistent with the glycation mechanism for complications. Others have reported a relationship between the G-gap and retinopathy and nephropathy (2,25,26). Cohen et al. (2) suggested that the G-gap increased the risk of more advanced nephropathy 2.9-fold. Rodríguez-Segade et al. (25) studied 2,314 patients with type 2 diabetes for a mean of 6.5 years, dividing the cohort into tertiles based on the average of all individual G-gaps, and showed that the mean G-gap predicts the progression of nephropathy. In an alternative fructosamine-based approach, the hemoglobin glycation index (HGI), the G-gap was calculated as the difference between the measured HbA1c minus an HbA1c predicted from date-matched mean blood glucose estimations (3). In a study by McCarter et al. (26) analyzing the data from DCCT, HGI was shown to be a significant predictor of retinopathy and nephropathy. To our knowledge, ours is the first published study confirming some potential association between the G-gap and macrovascular disease.

With a relationship between G-gap and diabetes vascular complications, mortality would be expected to follow a similar pattern. This was not so. Adjusted all-cause mortality was higher both in the negative and positive G-gap groups. The limitations of our study are manifest with it being a cross-sectional, retrospective study that was neither designed nor powered to address mortality, and we have no data on cause of death, noting that diabetes is associated with increased mortality from both vascular and a variety of nonvascular causes (27). It would be tempting to conjecture on reasons why a positive G-gap might be associated with mortality, given the macrovascular association, but we can offer no true explanation in light of the overall effect. There are no previous published reports of any relationship between the G-gap and mortality.

The long-term follow-up of the UK Prospective Diabetes Study (UKPDS) cohort suggested some benefit for macrovascular outcome and mortality with lower HbA1c levels (28), but the conclusion of other studies and meta-analyses has demonstrated little or no impact of HbA1c on either macrovascular events or mortality (29–34). The ACCORD trial of intensification of therapy to a target HbA1c <6.0% (42 mmol/mol) stands out as having led to increased mortality with tighter glycemic targets for uncertain reasons (35). In a retrospective cohort study using the U.K. General Practice Research Database, Currie et al. (36) showed increased risk of all-cause mortality with both lower and higher HbA1c levels proposing a U-shaped association with the lowest risk at an HbA1c level of 7.5% (58 mmol/mol). Given the general failure to link HbA1c levels with mortality outcomes, our observation of an increased prevalence of a negative G-gap at lower HbA1c levels and of a positive G-gap at higher HbA1c levels, both associated with adverse mortality outcomes in a U-shaped pattern that mirrors the observations of Curie et al. (36), clearly offers an avenue for further exploration.

It has been argued that the any association of the G-gap with outcomes, whether calculated from fructosamine or HGI, is a statistically spurious outcome of regression analysis with the anchor HbA1c value (15). In all other published methodologies of the ascertainment of the G-gap (2,23,25,26,37), an HbA1c equivalent from fructosamine or blood glucose data has been derived by regression analysis. Our methodology specifically avoids this. With our methodology, we have previously shown that over time and repeated measures, the G-gap remains consistent within subjects despite significant within-subject variations in HbA1c and fructosamine and that the variation away from HbA1c is larger than statistically expected by Altman-Bland analysis (1,19).

There is a great need to be cautious about the G-gap, and many caveats must be attached to this concept. As well as the concern that the G-gap may well be a spurious statistical phenomenon, there is concern about the use of fructosamine. Fructosamine represents the glycation of a number of proteins although predominantly albumin, the time frame of representation of glycemic attainment may be shorter than that of HbA1c (remembering...
that HbA1c glycation itself is most influenced by glucose levels over the preceding 30 days), the glycation product assessed is not as specific as defined for HbA1c, its glycation may mirror protein turnover rates and protein loss as proteinuria, and it is influenced by shorter time frame changes. Thus, subjects who tightened up on diet, lifestyle, and other treatment prior to their blood testing or those who had intercurrent illness with short-term deterioration could well have introduced a gap between HbA1c and fructosamine, which would have translated into a G-gap.

To counter this anxiety, it should be pointed out that in general, as confirmed in this study, many have shown a good relationship between HbA1c and fructosamine (1.2,25). Fructosamine is known to be well associated with preceding blood glucose levels (38). A concern relating to the possible association of fructosamine levels with proteinuria has been published to be significant (13). In our own regression analysis of fructosamine with multiple relevant factors, we show that, all in all, they account for no more than 20% of the variance in fructosamine, which is to say 80% of fructosamine is not in any association with any known influencing factor. Among that medley of associated factors as presented, UACR was the last entered variable being the statistically weakest independent association, with an \( r^2 \) progression of 0.002 thus representing only 0.2% of the accountable variance of fructosamine. In direct bivariate terms, the relationship of fructosamine to (log)UACR was nonsignificant (\( P > 0.4 \)). Although in end-stage renal failure there is well-known unreliability of HbA1c (indeed fructosamine may be the better estimate), it does not seem appropriate to extend these concerns into the G-gap outcomes in our cohort tested (39). Finally, we can clearly state that the relationship of G-gap to mortality was independent of proteinuria. That is to say that although proteinuria and protein turnover themselves may be associated with mortality and may influence fructosamine, the associations of G-gap to mortality are statistically independent of that as far as we can determine.

Thus, it seems that fructosamine is an acceptable measure of glycemia attainment but it cannot be considered a gold standard measure. In this regard, it would be the validation of the G-gap by blood glucose that would best reflect the deflection of HbA1c glycation. A study of the G-gap and the HGI has confirmed that the two indices are highly correlated and consistent (40).

It is important to stress that the G-gap is not truly independent of HbA1c since the G-gap is computed as the difference between a measured and a predicted HbA1c, and so independence is impossible. In that regard, any association of G-gap with outcomes such as mortality will always be difficult to dissect away from an association with glycemia. However, it would not be expected that a single point HbA1c would have any casual bearing on mortality. Furthermore, in studies that have linked HbA1c to mortality, if any link actually exists, the relationship is complex and the factors of linkage ill understood (41,42). In any case, the G-gap itself is not fully associated with glycemic control, as indicated by the weak correlation with HbA1c (\( r = 0.38, \quad P = 0.001 \), variance explained \( \left[ r^2 \right] = 14\% \)) and fructosamine (\( r = -0.33, \quad P = 0.001 \), variance explained = 11%). Finally, introducing HbA1c into the model in the Cox regression analysis had no association with all-cause mortality (\( P = 0.082 \)) and did not alter the significant association of G-gap with mortality.

In conclusion, evidence is mounting around the G-gap but significant caveats remain, and it may yet turn out to be a spurious phenomenon, especially since no defining mechanism is as yet proposed. We do now know that it appears to vary with certain demographic characteristics, it is consistent in direction over time, and, when positive, is associated with the key diabetes vascular complications in a manner coherent with one key underlying pathophysiological mechanism, protein glycation. We now further show an unexpected association of completely uncertain etiology with mortality with both a negative and a positive G-gap in a U-shaped relationship with no associated effect of the prevailing HbA1c.

Our paper confirms the reported G-gap association with retinopathy and nephropathy, and the findings for demographics, macrovascular disease, and mortality are previously unreported.

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**Table 2—Binary regression analysis showing the association of G-gap status and various other factors with adverse diabetes outcomes**

<table>
<thead>
<tr>
<th>Overall model ( (\chi^2) )</th>
<th>Any retinopathy</th>
<th>UACR &gt;10 mg/mmol</th>
<th>Any macrovascular</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-gap category</td>
<td>( P = 0.016 )</td>
<td>( P &lt; 0.001 )</td>
<td>( P = 0.28 )</td>
</tr>
<tr>
<td>Negative vs. neutral</td>
<td>0.84 (0.68–1.05), ( P = 0.12 )</td>
<td>0.93 (0.70–1.23), ( P = 0.61 )</td>
<td>0.96 (0.75–1.23), ( P = 0.74 )</td>
</tr>
<tr>
<td>Positive vs. neutral</td>
<td>1.24 (1.01–1.52), ( P = 0.039 )</td>
<td>1.55 (1.23–1.95), ( P &lt; 0.001 )</td>
<td>1.17 (0.95–1.45), ( P = 0.14 )</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.99 (0.98–0.99), ( P &lt; 0.001 )</td>
<td>1.02 (1.01–1.02), ( P &lt; 0.001 )</td>
<td>1.06 (1.05–1.07), ( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Sex (male vs. female)</td>
<td>1.26 (1.07–1.49), ( P = 0.006 )</td>
<td>1.41 (1.15–1.73), ( P = 0.001 )</td>
<td>1.55 (1.29–1.85), ( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>( P = 0.002 )</td>
<td>( P = 0.001 )</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Asian vs. Caucasian</td>
<td>1.35 (1.10–1.66), ( P = 0.004 )</td>
<td>1.59 (1.25–2.01), ( P &lt; 0.001 )</td>
<td>1.42 (1.14–1.76), ( P = 0.002 )</td>
</tr>
<tr>
<td>Black vs. Caucasian</td>
<td>1.45 (1.10–1.91), ( P = 0.008 )</td>
<td>1.21 (0.87–1.67), ( P = 0.25 )</td>
<td>0.61 (0.45–0.81), ( P = 0.001 )</td>
</tr>
<tr>
<td>Smoking (ever vs. never)</td>
<td>0.86 (0.72–1.02), ( P = 0.08 )</td>
<td>1.38 (1.12–1.71), ( P = 0.003 )</td>
<td>1.54 (1.27–1.85), ( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Diabetes type (type 2 vs. type 1)</td>
<td>1.31 (0.98–1.75), ( P = 0.07 )</td>
<td>1.11 (0.77–1.59), ( P = 0.57 )</td>
<td>2.05 (1.46–2.89), ( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>1.08 (1.07–1.09), ( P &lt; 0.001 )</td>
<td>1.02 (1.01–1.03), ( P = 0.001 )</td>
<td>1.02 (1.01–1.03), ( P = 0.001 )</td>
</tr>
<tr>
<td>On insulin (yes vs. no)</td>
<td>1.50 (1.24–1.81), ( P &lt; 0.001 )</td>
<td>1.03 (0.81–1.31), ( P = 0.84 )</td>
<td>1.97 (1.60–2.44), ( P &lt; 0.001 )</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>1.01 (1.00–1.02), ( P = 0.22 )</td>
<td>1.02 (1.01–1.04), ( P = 0.003 )</td>
<td>1.03 (1.01–1.04), ( P &lt; 0.001 )</td>
</tr>
</tbody>
</table>

*For continuous variables (age, diabetes duration, and BMI), the OR represents the change in risk per unit change in the variable (e.g., increase per year of age), whereas for categorical variables, it is with highest risk category.*
Cohort categorized according to G-gap status (for positive G-gap, n = 1,945; and negative G-gap, n = 686).

Figure 2—Cox regression analysis for cumulative survival from time of first testing of the whole cohort categorized according to G-gap status (for positive G-gap, n = 651; neutral G-gap, n = 1,945; and negative G-gap, n = 586).

Acknowledgments—This study was supported by a grant from the South Staffordshire Medical Foundation Trust, U.K.

No potential conflicts of interest relevant to this article were reported.

A.U.N. and B.M.S. researched the data, performed the statistical analysis, and wrote the draft of the manuscript. A.M.N. and P.B. supported the statistical analysis. All authors participated in data interpretation and commented on the draft of the manuscript and approved the final version. A.U.N. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors acknowledge help kindly provided by Dr. Alex Wright (University of Birmingham, Birmingham, U.K.) toward editing the final draft prior to its submission.

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