Association of Glycation Gap With Mortality and Vascular Complications in Diabetes

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OBJECTIVE—The "glycation gap" (G-gap), an essentially unproven concept, is an empiric measure of disagreement between HbA_{1c} 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 HbA_{1c} and a fructosamine-derived standardized predicted HbA_{1c} 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.

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The glycation gap (G-gap) refers to the potential deviation of glycated HbA_{1c} away from the other indirect estimate of blood glucose attainment such that it might read substantially lower or higher than expected (1–3). Glycated HbA_{1c} 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 HbA_{1c}, 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 HbA_{1c} variability, but any impact on HbA_{1c} glycation is unknown; although it seems unlikely that glycated HbA_{1c} is a substrate for this enzyme since it has been shown that there is no evidence that it plays any role in HbA_{1c} 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 HbA_{1c} 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 article 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 HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} testing were excluded.

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Retinopathy grading, microalbuminuria, and macrovascular risk

Digital retinal screening was in accordance with the English National Screening Program for Diabetic Retinopathy (ENSPDR) (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

HbA_{1c} International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) values were available only from 1 June 2009. Hence we have used the Diabetes Control and Complications Trial (DCCT)–aligned HbA_{1c} in our analysis. HbA_{1c} 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) were as follows: 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 HbA_{1c} of 5.7% (39 mmol/mol) and 9.5% (80 mmol/mol), respectively. Fructosamine was measured by nitrotetrazolium-blue reduction on a Roche Modular P analyzer (Roche Diagnostics Ltd., West Sussex, U.K.) using a Cobas kit 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 HbA_{1c} and the G-gap

As published (1), a predicted HbA_{1c} ($FHbA_{1c}$) was calculated from the simultaneously measured fructosamine standardized to the HbA_{1c} distribution according to the following equation: $FHbA_{1c}$ = {[(fructosamine − mean fructosamine)/SD fructosamine] × SD HbA_{1c}} + mean HbA_{1c}. The G-gap was the difference between the true HbA_{1c} and the fructosamine-derived standardized predicted $FHbA_{1c}$ (G-gap = HbA_{1c} − $FHbA_{1c}$). Importantly the $FHbA_{1c}$ was not derived from HbA_{1c} by correlation/regression methods. 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 the fructosamine-derived value. A negative G-gap denotes the true HbA_{1c} appearing to read lower than the $FHbA_{1c}$, and a positive G-gap denotes the true HbA_{1c} appearing to read higher than that predicted by fructosamine. Among those with a second paired HbA_{1c}-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), HbA_{1c} was associated significantly (r^2 = 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 (r^2 = 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 HbA_{1c} (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 = HbA_{1c} %) 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 χ^2 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 HbA_{1c}-fructosamine estimation to the time of death or study end point was 38 ± 16 months.

Table 1 shows the glycation estimates. The correlation between HbA_{1c} and fructosamine in the first HbA_{1c}-fructosamine pair is r = 0.75, $P < 0.001$ (n = 3,182). Nevertheless, the G-gap range demonstrates the substantial magnitude of variation between HbA_{1c} and $FHbA_{1c,}$ 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 HbA_{1c} quintile (χ^2 = 505.8, $P < 0.001$), noting the striking increase in negative G-gap status in the lowest HbA_{1c} quintile, whereas the positive G-gap prevalence was graded across ascending quintiles (Fig. 1). Repeat HbA_{1c}-fructosamine estimations were undertaken 11 ± 10 months after the first in 1,609 patients. There was a quadratic relationship (r^2 = 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).

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

G-gap category (HbA_{1c} %)	Negative (≤ −1)	Neutral (> −1 to < +1)	Positive (≥ +1)	*P*
Number (%)	586 (18)	1,945 (61)	651 (21)	
HbA_{1c} (%)	7.9 ± 1.8	8.2 ± 1.5	9.9 ± 2.0	$P < 0.001$
Fructosamine (μmol/L)	365 ± 91	301 ± 66	298 ± 75	$P < 0.001$
$FHbA_{1c}$ (%)	9.7 ± 2.1	8.2 ± 1.5	8.2 ± 1.7	$P < 0.001$
G-gap (HbA_{1c} %)	−1.8 ± 0.8	0.0 ± 0.5	+1.7 ± 0.8	$P < 0.001$
Age (years)	61 ± 18	66 ± 14	64 ± 13	$P < 0.001$
Sex (% male)	63	54	47	$P < 0.001$
Ethnicity (% white, Asian, black)	68, 6, 16	62, 28, 10	62, 32, 6	$P < 0.001$
Smoking status (% never, ex, current)	64, 27, 9	62, 29, 9	57, 28, 15	$P = 0.006$
Diabetes type (% type 2)	60	85	90	$P < 0.001$
Duration of diabetes (years)	20 ± 11	17 ± 9	16 ± 8	$P < 0.001$
On insulin (%)	76	68	76	$P < 0.001$
BMI (kg/m^2)	28.8 ± 5.6	31.8 ± 6.1	34.4 ± 7.4	$P < 0.001$
Systolic blood pressure (mmHg)	136 ± 21	138 ± 21	137 ± 12	$P = 0.09$
Diastolic blood pressure (mmHg)	73 ± 12	73 ± 12	74 ± 11	$P = 0.38$
Cholesterol (mmol/L)	4.4 ± 1.1	4.3 ± 1.0	4.4 ± 1.2	$P = 0.05$
Retinopathy (% any)	62	61	66	$P = 0.05$
UACR (% >10 mg/mmol)	15	17	24	$P < 0.001$
UACR (mg/mmol)	10 ± 56	12 ± 48	22 ± 70	$P < 0.001$
Serum creatinine (μmol/L)	93 ± 30	92 ± 29	87 ± 28	$P = 0.001$
eGFR (mL/min)	80 ± 27	76 ± 26	79 ± 27	$P = 0.003$
Macrovascular risk (% established)	24	30	33	$P = 0.001$
Vital status (number [%] dead)	87 (15)	196 (10)	108 (16)	$P < 0.001$

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], $P < 0.01$). See Supplementary Data online for Supplementary Table 1 and annotation.

Figure 2 clearly shows the differing mortality outcome between the groups ($P < 0.001$) categorized according to G-gap status (negative vs. neutral: OR 1.96 [95% CI 1.50–2.55], $P < 0.001$; positive vs. neutral: 2.02 [1.57–2.60], $P < 0.001$). Thus for mortality, in contrast to retinopathy, nephropathy, and macrovascular disease where the neutral and negative G-gap groups did not significantly differ from each other, both the negative and positive G-gap groups had a significantly worse outcome than the neutral group.

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

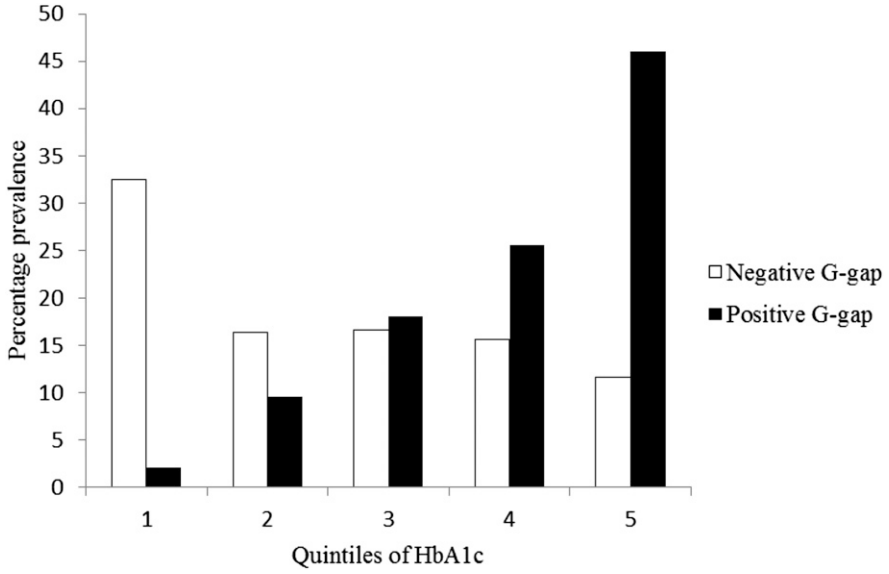

Figure 1—*The prevalence of negative and positive G-gap status by HbA_{1c} quintile ($\chi^2 = 505.8$, $P < 0.001$). The HbA_{1c} (mean [range]) for the quintiles of HbA_{1c}: 1) 6.4% (3.8–7.0), 2) 7.5% (7.1–7.8), 3) 8.3% (7.9–8.6), 4) 9.2% (8.7–9.7), and 5) 11.3% (9.8–19.0).*

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 HbA_{1c} 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 HbA_{1c} 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 non–fructosamine-based approach, the hemoglobin glycation index (HGI), the G-gap was calculated as the difference between the measured HbA_{1c} minus an HbA_{1c} 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 morality 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 HbA_{1c} levels (28), but the conclusion of other studies and meta-analyses has demonstrated little or no impact of HbA_{1c} on either macrovascular events or mortality (29–34). The ACCORD trial of intensification of therapy to a target HbA_{1c} <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 HbA_{1c} levels proposing a U-shaped association with the lowest risk at an HbA_{1c} level of 7.5% (58 mmol/mol). Given the general failure to link HbA_{1c} levels with mortality outcomes, our observation of an increased prevalence of a negative G-gap at lower HbA_{1c} levels and of a positive G-gap at higher HbA_{1c} levels, both associated with adverse mortality outcomes in a U-shaped pattern that mirrors the observations of Currie 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 HbA_{1c} value (15). In all other published methodologies of the ascertainment of the G-gap (2,3,25,26,37), an HbA_{1c} 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 HbA_{1c} and fructosamine and that the variation away from HbA_{1c} 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 HbA_{1c} (remembering

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

	Any retinopathy	UACR >10 mg/mmol	Any macrovascular
Overall model (χ^2)	324.5, $P < 0.001$	81.6, $P < 0.001$	481.1, $P < 0.001$
	OR (95% CI)* for individual factors		
G-gap category	$P = 0.016$	$P < 0.001$	$P = 0.28$
Negative vs. neutral	0.84 (0.68–1.05), $P = 0.12$	0.93 (0.70–1.23), $P = 0.61$	0.96 (0.75–1.23), $P = 0.74$
Positive vs. neutral	1.24 (1.01–1.52), $P = 0.039$	1.55 (1.23–1.95), $P < 0.001$	1.17 (0.95–1.45), $P = 0.14$
Age (years)	0.99 (0.98–0.99), $P < 0.001$	1.02 (1.01–1.02), $P < 0.001$	1.06 (1.05–1.07), $P < 0.001$
Sex (male vs. female)	1.26 (1.07–1.49), $P = 0.006$	1.41 (1.15–1.73), $P = 0.001$	1.55 (1.29–1.85), $P < 0.001$
Ethnicity	$P = 0.002$	$P = 0.001$	$P < 0.001$
Asian vs. Caucasian	1.35 (1.10–1.66), $P = 0.004$	1.59 (1.25–2.01), $P < 0.001$	1.42 (1.14–1.76), $P = 0.002$
Black vs. Caucasian	1.45 (1.10–1.91), $P = 0.008$	1.21 (0.87–1.67), $P = 0.25$	0.61 (0.45–0.81), $P = 0.001$
Smoking (ever vs. never)	0.86 (0.72–1.02), $P = 0.08$	1.38 (1.12–1.71), $P = 0.003$	1.54 (1.27–1.85), $P < 0.001$
Diabetes type (type 2 vs. type 1)	1.31 (0.98–1.75), $P = 0.07$	1.11 (0.77–1.59), $P = 0.57$	2.05 (1.46–2.89), $P < 0.001$
Diabetes duration (years)	1.08 (1.07–1.09), $P < 0.001$	1.02 (1.01–1.03), $P = 0.001$	1.02 (1.01–1.03), $P = 0.001$
On insulin (yes vs. no)	1.50 (1.24–1.81), $P < 0.001$	1.03 (0.81–1.31), $P = 0.84$	1.97 (1.60–2.44), $P < 0.001$
BMI (kg/m^2)	1.01 (1.00–1.02), $P = 0.22$	1.02 (1.01–1.04), $P = 0.003$	1.03 (1.01–1.04), $P < 0.001$

*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.

that HbA_{1c} glycation itself is most influenced by glucose levels over the preceding 30 days), the glycation product assessed is not as specific as defined for HbA_{1c}, 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 HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} (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 HbA_{1c} 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 HbA_{1c} since the G-gap is computed as the difference between a measured and a predicted HbA_{1c}, 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 HbA_{1c} would have any casual bearing on mortality. Furthermore, in studies that have linked HbA_{1c} 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 HbA_{1c} ($r = 0.38$, $P = 0.001$, variance explained [r^2] = 14%) and fructosamine ($r = -0.33$, $P = 0.001$, variance explained = 11%). Finally, introducing HbA_{1c} 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, it 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 HbA_{1c}.

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

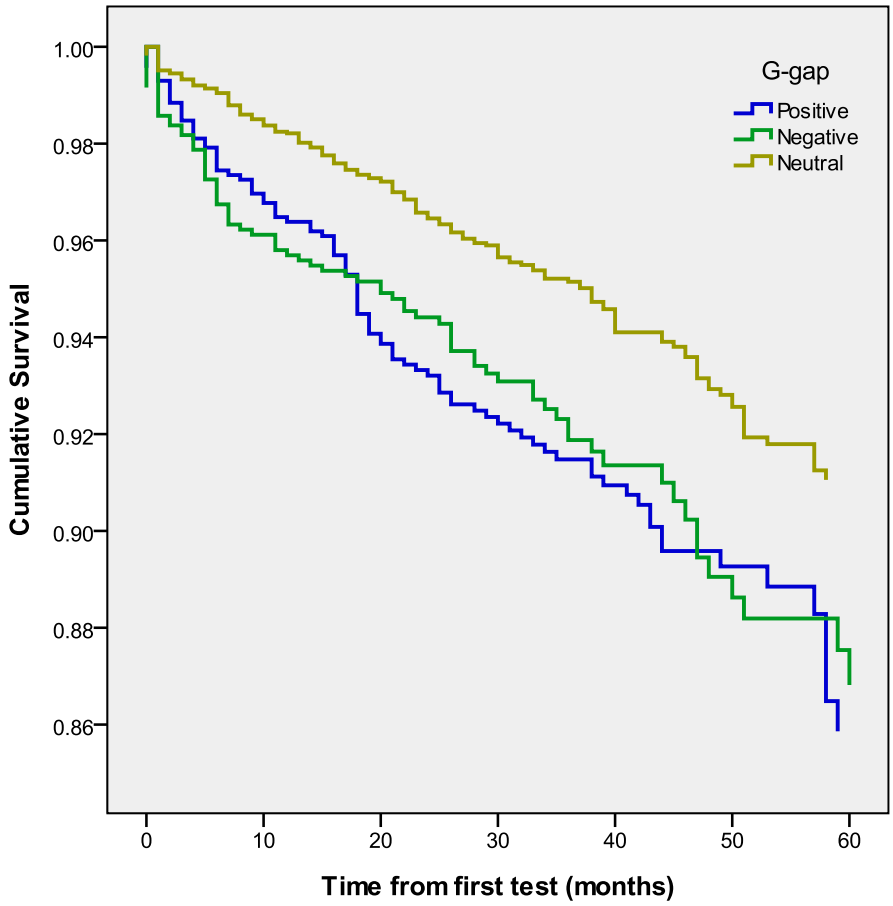

Figure 2—*Cox regression analysis for cumulative survival from time of first testing of the whole cohort categorized according to G-gap status (for positive G-gap,* n = *651; neutral G-gap,* n = *1,945; and negative G-gap,* n = *586).*

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References

1. Nayak AU, Holland MR, Macdonald DR, Nevill A, Singh BM. Evidence for consistency of the glycation gap in diabetes. Diabetes Care 2011;34:1712–1716
2. Cohen RM, Holmes YR, Chenier TC, Joiner CH. Discordance between HbA_{1c} and fructosamine: evidence for a glycosylation gap and its relation to diabetic nephropathy. Diabetes Care 2003;26:163–167
3. Hempe JM, Gomez R, McCarter RJ Jr, Chalew SA. High and low hemoglobin glycation phenotypes in type 1 diabetes: a challenge for interpretation of glycemic control. J Diabetes Complications 2002;16:313–320
4. Hudson PR, Child DF, Jones H, Williams CP. Differences in rates of glycation (glycation index) may significantly affect individual HbA_{1c} results in type 1 diabetes. Ann Clin Biochem 1999;36:451–459
5. Gould BJ, Davie SJ, Yudkin JS. Investigation of the mechanism underlying the variability of glycated haemoglobin in non-diabetic subjects not related to glycaemia. Clin Chim Acta 1997;260:49–64
6. Dagogo-Jack S. Pitfalls in the use of HbA_{1c} as a diagnostic test: the ethnic conundrum Nat Rev Endocrinol 2010;6:589–593
7. Cohen RM, Franco RS, Khera PK, et al. Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA_{1c}. Blood 2008;112:4284–4291
8. Khera PK, Joiner CH, Carruthers A, et al. Evidence for interindividual heterogeneity in the glucose gradient across the human red blood cell membrane and its relationship to hemoglobin glycation. Diabetes 2008;57:2445–2452
9. Soranzo N, Sanna S, Wheeler E, et al. Common variants at 10 genomic loci influence hemoglobin A_{1c} levels via glycemic and nonglycemic pathways. Diabetes 2010;59:3229–3239
10. Delpierrre G, Vertommen D, Communi D, Rider MH, Van Schaftingen E. Identification of fructosamine residues deglycated by fructosamine-3-kinase in human hemoglobin. J Biol Chem 2004;279:27613–27620
11. Mohás M, Kisfali P, Baricza E, et al. A polymorphism within the fructosamine-3-kinase gene is associated with HbA_{1c} levels and the onset of type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 2010;118:209–212
12. Delpierre G, Veiga-da-Cunha M, Vertommen D, Buysschaert M, Van Schaftingen E. Variability in erythrocyte fructosamine 3-kinase activity in humans correlates with polymorphisms in the FN3K gene and impacts on haemoglobin glycation at specific sites. Diabetes Metab 2006;32:31–39
13. Chan JC, Yeung VT, Cheung CK, Swaminathan R, Cockram CS. The interrelationships between albuminuria, plasma albumin concentration and indices of glycaemic control in non-insulin-dependent diabetes mellitus. Clin Chim Acta 1992;210:179–185
14. Perry HM 3rd, Morley JE, Miller DK. Serum fructosamine and obesity. Diabetes Care 1999;22:1596–1597
15. Sacks DB, Nathan DM, Lachin JM. Gaps in the glycation gap hypothesis. Clin Chem 2011;57:150–152
16. Retinopathy grading recommendations by the English National Screening Programme for Diabetic Retinopathy (ENSPDR). Available from http://www.retinalscreening.nhs.uk/pages/). Accessed 27 September 2011
17. Marshall SM. Screening for microalbuminuria: which measurement? Diabet Med 1991;8:706–711
18. Schleicher ED, Mayer R, Wagner EM, Gerbitz KD. Is serum fructosamine assay specific for determination of glycated serum protein? Clin Chem 1988;34:320–323
19. Macdonald DR, Hanson AM, Holland MR, Singh BM. Clinical impact of variability in HbA_{1c} as assessed by simultaneously measuring fructosamine and use of error grid analysis. Ann Clin Biochem 2008;45:421–425
20. Cohen RM, Snieder H, Lindsell CJ, et al. Evidence for independent heritability of

the glycation gap (glycosylation gap) fraction of HbA_{1c} in nondiabetic twins. Diabetes Care 2006;29:1739–1743
21. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813–820
22. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998;352: 837–853
23. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329: 977–986
24. Goh SY, Cooper ME. Clinical review: the role of advanced glycation end products in progression and complications of diabetes. J Clin Endocrinol Metab 2008;93: 1143–1152
25. Rodríguez-Segade S, Rodríguez J, Cabezas-Agricola JM, Casanueva FF, Camiña F. Progression of nephropathy in type 2 diabetes: the glycation gap is a significant predictor after adjustment for glycohemoglobin (HbA_{1c}). Clin Chem 2011;57:264–271
26. McCarter RJ, Hempe JM, Gomez R, Chalew SA. Biological variation in HbA1c predicts risk of retinopathy and nephropathy in type 1 diabetes. Diabetes Care 2004;27:1259–1264
27. The Emerging Risk Factors Collaboration, Seshasai SR, Kaptoge S, Thompson A, et al. Diabetes mellitus, fasting glucose, and risk of cause-specific death. N Engl J Med 2011;364:829–841
28. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HAW. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2008;359:1577–1589
29. Ray KK, Seshasai SR, Wijesuriya S, et al. Effect of intensive control of glucose on cardiovascular outcomes and death in patients with diabetes mellitus: a meta-analysis of randomised controlled trials. Lancet 2009;373:1765–1772
30. Boussageon R, Bejan-Angoulvant T, Saadatian-Elahi M, et al. Effect of intensive glucose lowering treatment on all cause mortality, cardiovascular death, and microvascular events in type 2 diabetes: meta-analysis of randomised controlled trials. BMJ 2011;343:d4169
31. Kelly TN, Bazzano LA, Fonseca VA, Thethi TK, Reynolds K, He J. Systematic review: glucose control and cardiovascular disease in type 2 diabetes. Ann Intern Med 2009;151:394–403
32. Brown A, Reynolds LR, Bruemmer D. Intensive glycemic control and cardiovascular disease: an update. Nat Rev Cardiol 2010;7:369–375
33. Duckworth W, Abraira C, Moritz T, et al.; VADT Investigators. Glucose control and vascular complications in veterans with type 2 diabetes. N Engl J Med 2009;360: 129–139
34. ADVANCE Collaborative Group, Patel A, MacMahon S, Chalmers J, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med 2008;358:2560–2572
35. ACCORD Study Group; Gerstein HC, Miller ME, Genuth S, et al. Long-term effects of intensive glucose lowering on cardiovascular outcomes. N Engl J Med 2011;364:818–828
36. Currie CJ, Peters JR, Tynan A, et al. Survival as a function of $HbA(_{1c})$ in people with type 2 diabetes: a retrospective cohort study. Lancet 2010;375:481–489
37. Lachin JM, Genuth S, Nathan DM, Rutledge BN. The hemoglobin glycation index is not an independent predictor of the risk of microvascular complications in the Diabetes Control and Complications Trial. Diabetes 2007;56:1913–1921
38. Baker JR, Metcalf PA, Holdaway IM, Johnson RN. Serum fructosamine concentration as measure of blood glucose control in type I (insulin dependent) diabetes mellitus. Br Med J (Clin Res Ed) 1985;290:352–355
39. Vos FE, Schollum JB, Coulter CV, Manning PJ, Duffull SB, Walker RJ. Assessment of markers of glycaemic control in diabetic patients with chronic kidney disease using continuous glucose monitoring. Nephrology (Carlton) 2012;17:182–188
40. Chalew SA, McCarter RJ, Thomas J, Thomson JL, Hempe JM. A comparison of the glycosylation gap and hemoglobin glycation index in patients with diabetes. J Diabetes Complications 2005;19:218–222
41. Zoungas S, Chalmers J, Ninomiya T, et al.; ADVANCE Collaborative Group. Association of HbA_{1c} levels with vascular complications and death in patients with type 2 diabetes: evidence of glycaemic thresholds. Diabetologia 2012;55:636–643
42. Rutter MK. Low HbA_{1c} and mortality: causation and confounding. Diabetologia 2012;55:2307–2311