Glycation Gap Is Associated With Macroproteinuria but Not With Other Complications in Patients With Type 2 Diabetes

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OBJECTIVE—We investigated whether glycation gap (G-Gap), an index of intracellular glycation of proteins, was associated with diabetes complications.

RESEARCH DESIGN AND METHODS—We measured concomitantly HbA_{1c} and fructosamine in 925 patients with type 2 diabetes to calculate the G-Gap, defined as the difference between measured HbA_{1c}, and fructosamine-based predicted HbA_{1c}. Patients were explored for retinopathy, nephropathy, peripheral neuropathy, cardiac autonomic neuropathy (n = 512), and silent myocardial ischemia (n = 506).

RESULTS—Macroproteinuria was the only complication that was associated with G-Gap (prevalence in the first, second, and third tertile of G-Gap: 2.9, 6.2, and 11.0%, respectively; $P < 0.001$). The G-Gap was higher in patients with macroproteinuria than in those without (1.06 ± 1.62 vs. 0.03 ± 1.30%; $P < 0.0001$). Because HbA_{1c} was associated with both G-Gap (HbA_{1c} 7.0 ± 1.4, 7.9 ± 1.4, and 10.1 ± 1.8% in the first, second, and third G-Gap tertile, respectively; $P < 0.0001$) and macroproteinuria (HbA_{1c} 8.8 ± 2.2% if macroproteinuria, 8.3 ± 2.0% if none; $P < 0.05$), and because it could have been a confounder, we matched 54 patients with macroproteinuria and 200 patients without for HbA_{1c}. Because macroproteinuria was associated with lower serum albumin and fructosamine levels, which might account for higher G-Gap, we calculated in this subpopulation albumin-indexed fructosamine and G-Gap; macroproteinuria was independently associated with male sex (odds ratio [OR] 3.2 [95% CI 1.5–6.7]; $P < 0.01$), hypertension (2.9 [1.1–7.5]; $P < 0.05$), and the third tertile of albumin-indexed G-Gap (2.3 [1.1–4.4]; $P < 0.05$) in multivariate analysis.

CONCLUSIONS—In type 2 diabetic patients, G-Gap was associated with macroproteinuria, independently of HbA_{1c}, albumin levels, and confounding factors, suggesting a specific role of intracellular glycation susceptibility on kidney glomerular changes.

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Protein glycation is involved in diabetes complications, and glycated hemoglobin (HbA_{1c}) level is associated with diabetes complications. Because glycation starts with glucose, it has been assumed that mean blood glucose is at the beginning of this association. However, even if there has been a close correlation between HbA_{1c} and mean blood glucose level over the previous 3 months, one-fifth (1) to one-third (2) of HbA_{1c} variance cannot be explained by mean blood glucose. Nonglycemic determinants of HbA_{1c} actually also could account for diabetes complications. One of the involved mechanisms might be an interindividual variation in the intracellular glycation of proteins, independently of glucose levels: the higher the protein glycation in target tissues (such as retina, kidney, neuronal tissues, and vessels), the more prevalent the tissue damages would be.

Some indexes have been developed to estimate nonglycemic determinants of HbA_{1c}: hemoglobin glycation index is the difference between observed HbA_{1c} and the value calculated from its regression with mean plasma glucose (3), and glycation gap (G-Gap) (previously called glycosylation gap) is the difference between observed HbA_{1c} and the value calculated from its regression with fructosamine (4). There are several advantages to consider fructosamine rather than mean blood glucose to evaluate nonglycemic determinants of HbA_{1c}. First, fructosamine level is more stable than glucose itself. Second, fructosamine represents the 2-week blood glucose exposure, whereas frequent 7-point blood glucose profiles or continuous blood glucose monitoring is required to evaluate mean glucose. Moreover, fructosamine, unlike mean blood glucose, can be used to compare protein glycation in the extracellular space (fructosamine) and in the intracellular space (HbA_{1c} in red cells and, by assumption, in target tissues).

G-Gap has been shown to be consistent over time in type 2 (5,6) and type 1 diabetic patients (4,6). In a study including 40 patients with type 1 diabetes for >15 years, a 1% increase in G-Gap was associated with a 2.9-fold greater frequency of progression in the nephropathy stage. The data demonstrated that nephropathy correlated better with G-Gap than with HbA_{1c} or fructosamine alone (4). Furthermore, it recently has been shown that G-Gap predicted the progression of nephropathy in type 2 diabetic patients independently of fructosamine, even after adjustment for HbA_{1c} (5). Nevertheless, in type 1 diabetes, controversial

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data have been published on G-Gap and retinopathy (7), whereas in type 2 diabetes there has been no report on the relations between G-Gap and complications outside of nephropathy.

Thus, the aim of our study was to evaluate, in a large series of type 2 diabetic patients, the potential association between G-Gap and the presence of nephropathy, retinopathy, neuropathy, or silent myocardial ischemia independently of glycemic control.

RESEARCH DESIGN AND METHODS

Participants

The cohort included 925 adult inpatients with type 2 diabetes who had been referred to the Diabetes Department of Jean Verdier Hospital (Bondy, France) between 1994 and 2011. None had been admitted for an acute disease or had recently modified lifestyle or treatment. All of them had fructosamine, HbA_{1c}, and creatininemia measurements. To avoid any influence on fructosamine measurement (8), we excluded individuals with renal failure (creatinine clearance <60 mL/min) (9). The patients had no known hemoglobinopathy or erythrocyte disorder.

Nephropathy was defined as incipient nephropathy or overt macroproteinuria. Incipient nephropathy was defined as urinary albumin excretion rate between 30 and 299 mg over 24 h, and macroproteinuria was defined as urinary protein excretion rate ≥300 mg over 24 h on at least two measurements. Diabetic retinopathy was graded according to the Early Treatment of Diabetic Retinopathy Study severity scale and defined as absent or present. The diagnosis of peripheral neuropathy was based on the presence of any two or more of the following: neuropathic symptoms; decreased distal sensation; or decreased or absent ankle reflexes. Cardiac autonomic neuropathy was assessed in a subgroup of 512 patients using the following three tests as recommended (10): Valsalva; deep breathing; and lying to standing. Age was taken into account for test interpretation as previously described (11). Cardiac autonomic neuropathy was defined by at least two abnormal tests.

A subset of 506 patients was screened for silent myocardial ischemia. These patients had no cardiac symptoms, no history of coronary artery disease, a normal 12-lead resting ECG, and at least one of the following additional cardiovascular risk factors: dyslipidemia (total cholesterol >6.5 mmol/L or LDL cholesterol >4.1 mmol/L or both, HDL cholesterol <0.9 mmol/L, triglycerides >2.3 mmol/L or lipid-lowering medication or both); hypertension (systolic and diastolic blood pressure ≥140/90 mmHg or antihypertensive therapy); smoking; nephropathy; family history of premature coronary artery disease; and peripheral or carotid occlusive arterial disease. As previously reported (12–15), each patient underwent a ^{201}Tl myocardial scintigraphy after an ECG stress test or a pharmacological stress test (dipyridamole injection) or both. An ECG stress test was performed in the patients who could exercise on a bicycle ergometer and were expected to have an interpretable exercise ECG. When the patient was unable to exercise or when the ECG stress test result was indeterminate, a pharmacological stress test using dipyridamole was performed. Silent myocardial ischemia was defined as an abnormal ECG stress test or an abnormal myocardial scintigraphy imaging, i.e., defects in at least three out of 17 segmental regions, or both.

Biochemical measurements

All the other measurements were performed on the second day of hospitalization, at fasting. Glucose was measured on venous plasma by the glucose oxidase method (colorimetry, Kone Optima; Thermolab System, Paris La Défense, France). HbA_{1c} measurement was based on a turbidimetric inhibition immunoassay principle and total hemoglobin was measured using a modified alkaline hematin reaction (Dimension Technology; Siemens Healthcare Diagnostics). The intra-assay and interassay coefficients of variation were, respectively, 1.3 and 1.2% for a normal blood sample (mean HbA_{1c} 5.3%) and 1.5 and 2.3% for an elevated HbA_{1c} level (mean HbA_{1c} 8.6%). Reliability was tested daily using internal controls (low and high levels of a Biorad control). The same HbA_{1c} measurement technology was performed throughout the period of inclusion (16). Fructosamine was measured by the nitroblue tetrazolium colorimetric procedure based on the reducing ability of fructosamine in alkaline solution (COBAS; Roche Diagnostics Gmbh, Penzberg, Germany) (17). The intra-assay and interassay coefficients of variation were 1.2 and 1.6%, respectively.

The following measurements also were performed: creatinine and serum albumin (colorimetric assay, COBAS; Roche Diagnostics); urinary albumin excretion rate (mean of two measurements, laser immunonephelometry, BN100; Dade-Behring); total cholesterol; HDL cholesterol; and triglycerides (enzymatic colorimetry, Hitachi 912; Roche Diagnostic, Meylan, France). LDL cholesterol was calculated using the Friedwald formula. The accuracy of all the methods used was evaluated biannually by national (external) quality-control surveys.

Statistical analyses

Continuous variables were expressed as means ± SD values and compared by one-way ANOVA or the Mann-Whitney *U* test as adequate. The significance of differences in proportions was tested with the χ^2 test. Logistic regression was used for multivariate analyses based on a model including the factors that were associated with a diabetes complication with a $P < 0.10$ in univariate analyses. Because HbA_{1c} and G-Gap were highly correlated, they could not be entered together in the logistic regression models. Therefore, we matched patients with or without each complication for HbA_{1c} to evaluate the potential association of this complication with G-Gap, independently of HbA_{1c}. Statistical analyses were performed using SPSS software (SPSS, Chicago, IL). $P = 0.05$ was considered for statistical significance.

RESULTS

Patient characteristics

The main clinical and biological characteristics of our population are reported in Table 1. The prevalence of retinopathy was 37.1%, prevalence of nephropathy was 28.2%, including macroproteinuria 8.5%, prevalence of peripheral neuropathy was 46.7%, and prevalence of cardiac autonomic neuropathy was 30.9%. Silent myocardial ischemia was found in 148 patients (29.2%).

Parameters associated with a low or a high G-Gap

G-Gap was calculated as the difference between measured HbA_{1c} and HbA_{1c} predicted from fructosamine based on the HbA_{1c}–fructosamine regression equation (4–6): predicted $HbA_{1c} = 0.020 \times$ fructosamine + 2.05 ($r = 0.731$; $P < 0.0001$) (Fig. 1). The patients then were separated by G-Gap tertiles (Fig. 1 and Table 1). Increasing G-Gap tertiles were positively associated with female sex, BMI, the

Table 1—*Characteristics of the patients by glycation gap tertiles*

	Total $n = 925$	G-Gap tertile 1 $n = 308$	G-Gap tertile 2 $n = 309$	G-Gap tertile 3 $n = 308$	P
Age (years)	57.8 ± 9.3	57.7 ± 9.6	58.7 ± 9.3	57.1 ± 8.9	0.08
Sex (male/female)	493/432	185/123	155/154	153/155	<0.05
BMI (kg/m^2)	30.9 ± 6.2	29.4 ± 5.5	31.5 ± 6.4	31.9 ± 6.5	<0.0001
Diabetes duration (years)	11.7 ± 8.0	11.2 ± 8.2	12.3 ± 8.6	11.6 ± 7.1	NS
Hypertension (%)	632 (69.1)	203 (67.4)	215 (69.6)	214 (70.4)	NS
Antihypertensive therapy (%)	521 (57.4)	164 (55.0)	178 (57.8)	179 (59.5)	NS
Dyslipidemia (%)	540 (59.2)	159 (53.0)	184 (59.9)	197 (64.6)	<0.05
Lipid-lowering treatment (%)	353 (38.5)	103 (34.0)	123 (39.9)	127 (41.5)	NS
Diabetes complications					
Retinopathy (%)	332 (37.1)	102 (34.6)	102 (34.1)	128 (42.5)	0.057
Nephropathy (%)	239 (28.2)	64 (23.1)	81 (28.0)	94 (33.2)	<0.05
Macroproteinuria (%)	57 (6.7)	8 (2.9)	18 (6.2)	31 (11.0)	0.001
Creatinine clearance (mL/min)	105.9 ± 32.7	100.4 ± 29.6	105.2 ± 34.1	112.0 ± 33.4	<0.0001
UAER (mg/24 h)	102 ± 359	52 ± 166	73 ± 199	181 ± 558	<0.0001
Peripheral neuropathy (%)	425 (46.7)	127 (41.8)	141 (46.8)	157 (51.3)	0.06
Silent myocardial ischemia (%)	148/506 (29.2)	42/139 (30.2)	52/159 (32.7)	54/208 (26.0)	NS
Cardiac autonomic neuropathy (%)	158/512 (30.9)	56/183 (30.6)	46/166 (27.7)	56/163 (34.4)	NS
Biological parameters					
G-Gap (%)	0.08 ± 1.37	−1.25 ± 0.70	−0.10 ± 0.29	1.59 ± 1.00	<0.0001
HbA_{1c} (%)	8.3 ± 2.0	7.0 ± 1.4	7.9 ± 1.4	10.1 ± 1.8	<0.0001
Fructosamine (μmol/L)	310 ± 72	311 ± 77	296 ± 68	322 ± 70	<0.0001
Fasting plasma glucose (mmol/L)	8.1 ± 3.2	7.8 ± 3.2	7.8 ± 3.0	8.6 ± 3.3	<0.01
Postprandial glucose (mmol/L)	11.3 ± 4.2	10.7 ± 4.1	10.9 ± 3.9	12.3 ± 4.6	<0.0001

BP, blood pressure; UAER, urinary albumin excretion rate.

presence of dyslipidemia, HbA_{1c}, fructosamine, and fasting and postprandial glucose values. Regarding diabetes complications, increasing G-Gap tertiles were associated with nephropathy, urinary albumin excretion rate, and creatinine clearance, whereas no association with either retinopathy or peripheral neuropathy or cardiac autonomic neuropathy or silent myocardial ischemia was found (Table 1).

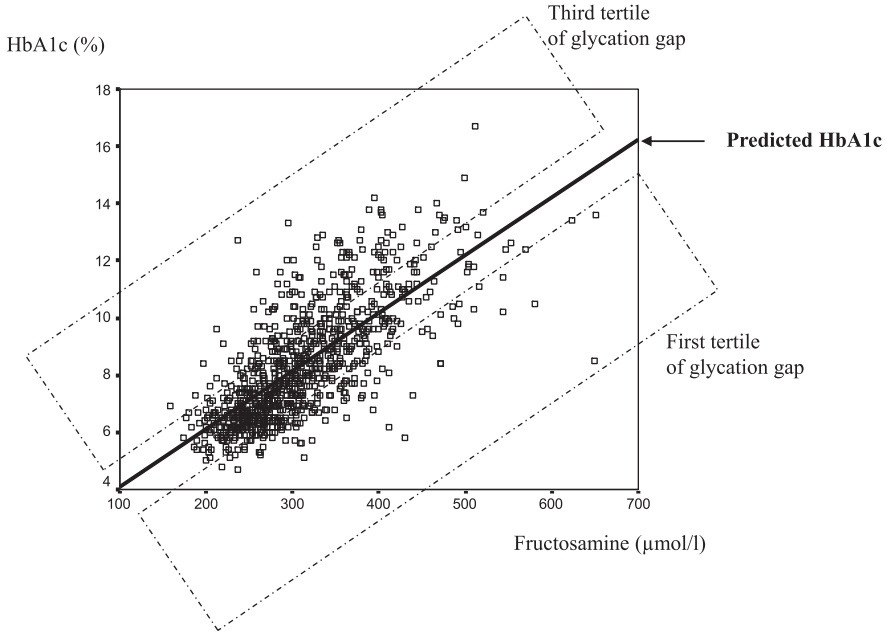

Figure 1—*Correlation between HbA_{1c} and fructosamine levels and repartition of the G-Gap tertiles.*

The association with nephropathy was driven by the association with macroproteinuria because there was no association between tertiles of G-Gap and incipient nephropathy was considered separately (prevalence of incipient nephropathy in the patients of the first, second, and third tertiles of G-Gap: 20.8, 23.2, and 25.0%, respectively; $P = 0.521$).

Parameters associated with nephropathy and macroproteinuria

Macroproteinuria was associated with male sex, diabetes duration, hypertension, dyslipidemia, G-Gap, HbA_{1c}, and fructosamine (Table 2). Compared with the first tertile of G-Gap considered as reference (2.9%), the prevalence of macroproteinuria did not differ significantly in the second tertile (6.2%; $P = 0.06$) and was higher in the third tertile (11.0%; odds ratio [OR] 4.1 [95% CI 1.9–9.2]; $P < 0.001$). G-Gap was greater in the patients with macroproteinuria than in those without (1.06 ± 1.62% vs. 0.03 ± 1.30%; $P < 0.0001$). Urinary albumin excretion rate (log) was correlated with G-Gap ($r = 0.141$; $P < 0.0001$). HbA_{1c} and G-Gap could not be considered together in multivariate analyses because HbA_{1c} was used to determine G-Gap.

Table 2—*Parameters associated with nephropathy and macroproteinuria*

	No nephropathy $n = 610$	Nephropathy $n = 23$	P	No macroproteinuria $n = 738$	Macroproteinuria $n = 57$	P
Age (years)	57.7 ± 9.0	58.6 ± 9.5	NS	58.1 ± 9.2	57.4 ± 8.4	NS
Sex (male/female)	288/321	161/78	<0.0001	381/357	43/14	<0.0001
BMI (kg/m^2)	30.7 ± 6.2	31.5 ± 6.5	0.09	31.0 ± 6.2	31.5 ± 6.6	NS
Diabetes duration (years)	11.3 ± 8.0	13.0 ± 7.6	0.01	11.6 ± 8.0	13.8 ± 7.3	0.05
Hypertension (%)	388 (64.8)	193 (81.1)	<0.0001	499 (68.1)	51 (89.5)	<0.0001
Dyslipidemia (%)	352 (58.7)	149 (62.9)	NS	457 (58.6)	44 (77.2)	<0.01
G-Gap (%)	0.01 ± 1.29	0.33 ± 1.52	<0.01	0.03 ± 1.30	1.1 ± 1.62	<0.0001
G-Gap (tertile 3) (%)	188 (30.9)	94 (39.3)	<0.05	234 (31.7)	31 (54.4)	<0.0001
HbA_{1c} (%)	8.2 ± 2.0	8.7 ± 2.1	<0.01	8.3 ± 2.0	8.8 ± 2.2	<0.05
Fructosamine (μmol/L)	307 ± 72	315 ± 72	NS	309 ± 72	284 ± 63	<0.05
Fasting plasma glucose (mmol/L)	8.0 ± 3.2	8.2 ± 2.9	NS	8.0 ± 3.2	8.0 ± 2.9	NS
Postprandial glucose (mmol/L)	11.3 ± 4.3	11.2 ± 4.0	NS	11.3 ± 4.2	10.4 ± 3.8	NS

In a multivariate analysis including male sex, diabetes duration, hypertension, dyslipidemia, and HbA_{1c} to explain macroproteinuria, all the parameters but diabetes duration and HbA_{1c} were independently associated with macroproteinuria. When male sex, diabetes duration, hypertension, dyslipidemia, and high G-Gap were considered, all but diabetes duration also were associated with macroproteinuria, including the third tertile of G-Gap (2.1 [1.4–3.0]; $P < 0.001$).

Nephropathy was associated with male sex, diabetes duration, hypertension, G-Gap, and HbA_{1c} (Table 2). Compared with the first tertile of G-Gap considered as reference (23.1%), the prevalence of nephropathy was similar in the second tertile (28.0%; $P = 0.18$) and higher in the third tertile (33.2%; OR 1.65 [95% CI 1.1–2.4]; $P < 0.01$). In multivariate analysis including male sex, diabetes duration, hypertension, BMI, and HbA_{1c} to explain nephropathy, all the parameters, including HbA_{1c} (1.1 [1.1–1.2]), were independently associated with nephropathy. When male sex, diabetes duration, hypertension, BMI, and a high G-Gap were considered, all were also associated with nephropathy, including the third tertile of G-Gap (1.6 [1.1–2.2]).

Parameters associated with diabetes complications after matching the patients for HbA_{1c}

Because HbA_{1c} and G-Gap are highly linked together, the association between G-Gap and nephropathy might be attributable to an association between HbA_{1c} and nephropathy. We therefore matched for HbA_{1c} 54 patients with macroproteinuria with 200 patients without macroproteinuria (Table 3). HbA_{1c} levels were similar in both groups. Macroproteinuria was associated with male sex, hypertension, lower albumin and fructosamine levels, higher G-Gap, and the third tertile of G-Gap (Table 3). Because of the potential influence of macroproteinuria on lower levels of serum albumin and fructosamine and, subsequently, on higher G-Gap levels, we corrected fructosamine values for variations in the concentrations of serum albumin (18) according to the following formula by Lamb et al. (19): albumin-indexed fructosamine = fructosamine (μmol/L) × 100/albumin (g/L). We then defined albumin-indexed predicted HbA_{1c} based on the HbA_{1c}/albumin-indexed fructosamine regression equation: 0.008 × albumin-indexed fructosamine + 2.109 ($r = 0.747$; $P < 0.0001$). Albumin-indexed G-Gap and its tertiles were then calculated as the difference between measured HbA_{1c} and albumin-indexed HbA_{1c}. Table 3 shows that macroproteinuria was associated with albumin-indexed G-Gap and its third tertile. Compared with the first tertile of albumin-indexed G-Gap considered as reference (11.5%), the prevalence of macroproteinuria was higher in the second (24.1%; OR 2.4 [95% CI 1.02–5.8]; $P < 0.05$) and third (30.8%; 3.4 [1.5–7.9]; $P < 0.01$) tertiles. In a multivariate analysis including sex, diabetes duration, hypertension, dyslipidemia, and the third tertile of albumin-indexed G-Gap, male sex, hypertension, and albumin-indexed G-Gap were independently associated with macroproteinuria (Table 3). When the same analysis was performed with albumin-indexed G-Gap rather than with its third tertile, albumin-indexed G-Gap also was independently associated with macroproteinuria (1.6 [1.2–2.1]; $P < 0.01$).

We also matched for HbA_{1c} 216 patients with nephropathy with 216 patients free of nephropathy (HbA_{1c} 8.5 ± 1.9% in both groups). G-Gap was similar in those with or without nephropathy (third tertile compared with first or second tertiles of G-Gap: prevalence of nephropathy 38.0 vs. 34.7%; $P = 0.548$).

There was a trend for an association between G-Gap tertiles and retinopathy or peripheral neuropathy (Table 1). Both complications also were associated with higher HbA_{1c} levels (retinopathy HbA_{1c} 8.7 ± 2.0% compared with no retinopathy HbA_{1c} 8.1 ± 1.2%; $P < 0.0001$; and neuropathy HbA_{1c} 8.6 ± 2.1% compared with no neuropathy HbA_{1c} 8.1 ± 1.9%; $P < 0.0001$). Therefore, we also matched for HbA_{1c} 260 patients with retinopathy and 260 without (HbA_{1c} in both groups 8.4 ± 1.8%). G-Gap was not associated with retinopathy (third tertile versus first or second tertiles of G-Gap: prevalence of retinopathy 50.5 vs. 49.7%; $P = 0.972$). We finally matched for HbA_{1c} 190 patients with peripheral neuropathy and 190 without (HbA_{1c} in both groups 8.3 ± 1.7%). G-Gap was not associated with peripheral neuropathy (third tertile versus first or second tertiles of G-Gap: prevalence of neuropathy 48.8 vs. 50.6%; $P = 0.827$).

CONCLUSIONS—We have shown here for the first time in a large cohort of patients with type 2 diabetes that a high G-Gap was associated with macroproteinuria but not with other complications.

Table 3—*Parameters associated with macroproteinuria in patients matched for HbA_{1c}*

Characteristics	No macroproteinuria $n = 200$	Macroproteinuria $n = 54$	Univariate P	Multivariate* OR	95% CI	P
Age (years)	57.7 ± 9.5	57.3 ± 8.6	NS			
Sex (male/female)	103/94	42/12	<0.0001	3.2	1.5–6.7	<0.01
BMI (kg/m^2)	31.1 ± 6.4	31.5 ± 6.6	NS			
Diabetes duration (years)	11.2 ± 7.4	13.4 ± 7.2	0.053	1.03	0.99–1.08	0.16
Hypertension (%)	139 (69.8)	48 (88.9)	<0.05	2.9	1.1–7.5	<0.05
Dyslipidemia (%)	123 (61.8)	41 (75.9)	0.06	2.0	0.9–4.2	0.07
Biological parameters						
G-Gap (%)	0.005 ± 1.212	0.864 ± 1.414	<0.0001			
G-Gap third tertile (%)	67 (33.5)	28 (51.9)	<0.05			
HbA_{1c} (%)	8.3 ± 1.7	8.5 ± 1.8	NS			
Fructosamine (μmol/L)	313 ± 65	279 ± 56	<0.0001			
Albumin (g/L)	41.5 ± 3.9	39.3 ± 4.5	<0.001			
Albumin-indexed fructosamine	747 ± 154	711 ± 156	0.145			
Albumin-indexed G-Gap (%)	0.14 ± 1.14	0.69 ± 1.12	<0.01			
Albumin-indexed G-Gap third tertile (%)	28 (17.8)	24 (30.8)	<0.05	2.3	1.1–4.4	<0.05
Fasting plasma glucose (mmol/L)	7.9 ± 2.7	8.0 ± 2.9	NS			
Postprandial glucose (mmol/L)	11.3 ± 3.8	10.5 ± 3.6	NS			

*Model including sex, diabetes duration, hypertension, dyslipidemia, and third tertile of albumin-indexed G-Gap.

This association appears to be independent of HbA_{1c}, albumin levels, and other confounders. Because G-Gap may reflect nonglycemic determinants of hemoglobin glycation, also called "glycability" (5), these results suggest that intracellular susceptibility for glycation in the glomerulus may be involved in the development of this diabetes complication.

McCarter et al. (20) previously have reported an association between hemoglobin glycation index and nephropathy among the type 1 diabetic patients of the Diabetes Control and Complications Trial. However, those data have been criticized because hemoglobin glycation index was by mathematical necessity linked with HbA_{1c}. Because a reanalysis of those data showed no significant influence of hemoglobin glycation index on the risk of nephropathy if HbA_{1c} was taken into account, this association was actually assumed to reflect an association between nephropathy and HbA_{1c} per se (21). Using G-Gap in a sample of 40 type 1 diabetic patients, Cohen et al. (4) reported an association between G-Gap and nephropathy. The regression between G-Gap and nephropathy was not altered when HbA_{1c}, fructosamine, and diabetes duration were included as factors in the analysis (4). In the current study, nephropathy was associated with HbA_{1c} and G-Gap, whereas HbA1c and G-Gap were positively and highly correlated. Therefore, we matched for HbA1c patients with or without macroproteinuria to avoid any HbA_{1c} effect. This procedure did not affect this association. Thus, these data demonstrate a correlation between a high G-Gap and prevalent macroproteinuria, independently of HbA_{1c} levels.

A high G-Gap may be a marker or predictor of nephropathy and also a consequence of nephropathy. Because other complications did not appear to be associated with G-Gap, specific mechanisms related to nephropathy might explain a high G-Gap. First, renal failure might explain between-patient differences in the mean age of circulating erythrocytes and, thus, variation in HbA_{1c} levels (22). Such a mechanism is unlikely to be involved in our study because we did not include patients with kidney failure. Furthermore, a similar prevalence of renal insufficiency was reported in the lowest and highest tertiles of G-Gap in a large cohort of type 2 diabetic patients (5). Second, glycated albumin, i.e., fructosamine, also may be less excreted than nonglycated albumin, especially in diabetic patients with macroproteinuria (23). With this hypothesis, fructosamine would be higher in case of proteinuria, which was not the case here. Furthermore, fructosamine was reported to remain unchanged with nephropathy stages in type 1 (4) and type 2 diabetes (5). Finally, the lower level of fructosamine reported here in the patients with macroproteinuria appeared to account for a higher G-Gap, but only partially, because the levels of HbA_{1c} were concomitantly higher. Third, urinary protein excretion may reduce serum albumin levels and, therefore, fructosamine levels (18). The association between G-Gap and nephropathy was driven in our study by macroproteinuria but not by incipient nephropathy, which could be in favor of this hypothesis. Such an effect was previously modeled considering body albumin daily synthesis and proteinuria, with a negative result (4). In our subpopulation with or without macroproteinuria matched for HbA_{1c}, we have shown that there was a 2.3-fold increased risk of macroproteinuria in the third tertile of albumin-indexed G-Gap in the multivariate analysis considering the other confounders, i.e., male sex, hypertension, dyslipidemia, and diabetes duration. Therefore, urinary protein loss may contribute to a lower fructosamine level and, therefore, a higher G-Gap; however, this is likely to be insufficient to explain by itself the association between macroproteinuria and G-Gap level.

The prospective study by Rodríguez-Segade et al. (5) provides arguments for a role played by G-Gap in the pathophysiology of nephropathy. They reported in a cohort of 2,314 type 2 diabetic patients with a 6.5-year follow-up that G-Gap predicted the progression of nephropathy, independently of fructosamine, even after

adjustment of HbA_{1c} (5). In that study, Cox regression analyses failed to show if HbA_{1c} or G-Gap was the best predictor, and we think that the associations of both are important. Because hemoglobin is an intracellular protein, and because fructosamine reflects extracellular proteins, a high G-Gap is considered as a marker of intracellular susceptibility for glycation. Such a mechanism therefore could contribute to nephropathy in type 1 and type 2 diabetes. Besides pathophysiological interest, G-Gap in clinical practice may be considered as a marker of proteinuria susceptibility and may lead to more preventive efforts in the patients with the highest G-Gap. This implies measuring, at least once, fructosamine together with HbA_{1c} to calculate the G-Gap.

Whether this process involves in a similar way tissues other than the renal glomerulus is unknown. There are only few data about other diabetes complications, and these data were reported only for type 1 diabetes. Regarding to retinopathy, the association between retinopathy and hemoglobin glycation index reported in patients from the Diabetes Control and Complications Trial (20) appeared to be attributable to HbA_{1c} (21). In a substudy from the Wisconsin Diabetes Registry Study, the probability of developing retinopathy increased with increasing G-Gap (7). We definitely did not find any independent association between retinopathy and G-Gap in this series of type 2 diabetic patients. Finally, we searched, for the first time, for an association between G-Gap and peripheral or cardiac autonomic neuropathy and silent myocardial ischemia in diabetic patients and failed to find any significant association. G-Gap therefore appears to play an important role only for overt nephropathy.

There are some limitations. The study was cross-sectional. We included only inpatients; therefore, the results are not necessarily generalizable to the diabetic population. Furthermore, we did not include patients with renal failure to improve the reliability of fructosamine measurement. G-Gap depends on HbA_{1c} and fructosamine levels; therefore, G-Gap variations may be explained by the different half-lives of its components. For example, a patient with improved glycemic control during the 2 weeks preceding measurement would decrease fructosamine level more than the HbA_{1c} value. As a consequence, the G-Gap level would be higher. This was not applicable to our patients because they were free of any acute condition at admission as checked with hospitalization code, and they had led a stable lifestyle during the weeks before admission. Furthermore, intraindividual G-Gap value has been reported to be consistent over time (4–6), with a genetic component (24).

To conclude, we demonstrate here for the first time in type 2 diabetic patients that overt nephropathy is associated with a high G-Gap, independently of HbA_{1c} levels and other confounders. G-Gap may be partly attributable to macroproteinuria, but data favor its involvement in renal disorders. A high G-Gap may represent tissue susceptibility to the risk associated with protein glycation and the formation of advanced glycation end products (25). This phenomenon appears to be restricted to the kidneys in type 2 diabetes.

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E.C. directed research and wrote the manuscript. I.B., C.C.-P., S.C., Y.J., and N.C. researched data and contributed to discussion. Q.C. researched data. M.T.N. researched data and performed the statistic analyses. P.V. directed research, contributed to discussion, and reviewed and edited manuscript. P.V. 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.

References

1. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ; A1c-Derived Average Glucose Study Group. Translating the A1C assay into estimated average glucose values. Diabetes Care 2008; 31:1473–1478
2. Rohlfing CL, Wiedmeyer HM, Little RR, England JD, Tennill A, Goldstein DE. Defining the relationship between plasma glucose and HbA(1c): analysis of glucose profiles and HbA(1c) in the Diabetes Control and Complications Trial. Diabetes Care 2002;25:275–278
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. Cohen RM, Holmes YR, Chenier TC, Joiner CH. Discordance between HbA1c and fructosamine: evidence for a glycosylation gap and its relation to diabetic nephropathy. Diabetes Care 2003;26:163–167
5. 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 (Hb A1c). Clin Chem 2011;57: 264–271
6. 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
7. Cohen RM, LeCaire TJ, Lindsell CJ, Smith EP, D'Alessio DJ. Relationship of prospective GHb to glycated serum proteins in incident diabetic retinopathy: implications of the glycation gap for mechanism of risk prediction. Diabetes Care 2008;31:151–153
8. Mittman N, Desiraju B, Fazil I, et al. Serum fructosamine versus glycosylated hemoglobin as an index of glycemic control, hospitalization, and infection in diabetic hemodialysis patients. Kidney Int Suppl 2010;117:S41–S45
9. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D; Modification of Diet in Renal Disease Study Group. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med 1999;130:461–470
10. Spallone V, Ziegler D, Freeman R, et al., on behalf of the Toronto Consensus Panel on Diabetic Neuropathy. Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. Diabetes Metab Res Rev 2011;27:639–653
11. Valensi P, Pariès J, Attali JR; French Group for Research and Study of Diabetic Neuropathy. Cardiac autonomic neuropathy in diabetic patients: influence of diabetes duration, obesity, and microangiopathic complications—the French multicenter study. Metabolism 2003;52:815–820
12. Cosson E, Guimfack M, Paries J, Paycha F, Attali JR, Valensi P. Prognosis for coronary stenoses in patients with diabetes and silent myocardial ischemia. Diabetes Care 2003;26:1313–1314
13. Cosson E, Guimfack M, Paries J, Paycha F, Attali JR, Valensi P. Are silent coronary stenoses predictable in diabetic patients and predictive of cardiovascular events? Diabetes Metab 2003;29:470–476
14. Cosson E, Nguyen MT, Pham I, Pontet M, Nitenberg A, Valensi P. N-terminal pro-B-type natriuretic peptide: an independent marker for coronary artery disease in asymptomatic diabetic patients. Diabet Med 2009;26:872–879
15. Cosson E, Nguyen MT, Chanu B, Balta S, Takbou K, Valensi P. The report of male gender and retinopathy status improves the current consensus guidelines for the screening of myocardial ischemia in asymptomatic type 2 diabetic patients.

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16. Cosson E, Nguyen MT, Hamo-Tchatchouang E, et al. What would be the outcome if the American Diabetes Association recommendations of 2010 had been followed in our practice in 1998-2006? Diabet Med 2011;28:567–574
17. Cefalu WT, Bell-Farrow AD, Petty M, Izlar C, Smith JA. Clinical validation of a second-generation fructosamine assay. Clin Chem 1991;37:1252–1256
18. Van Dieijen-Visser MP, Seynaeve C, Brombacher PJ. Influence of variations in albumin or total-protein concentration on serum fructosamine concentration. Clin Chem 1986;32:1610
19. Lamb E, Venton TR, Cattell WR, Dawnay A. Serum glycated albumin and fructosamine in renal dialysis patients. Nephron 1993;64:82–88
20. 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
21. 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
22. 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
23. Bent-Hansen L, Feldt-Rasmussen B, Kverneland A, Deckert T. Plasma disappearance of glycated and non-glycated albumin in type 1 (insulin-dependent) diabetes mellitus: evidence for charge dependent alterations of the plasma to lymph pathway. Diabetologia 1993;36:361–363
24. Cohen RM, Snieder H, Lindsell CJ, et al. Evidence for independent heritability of the glycation gap (glycosylation gap) fraction of HbA1c in nondiabetic twins. Diabetes Care 2006;29:1739–1743
25. Leslie RD, Cohen RM. Biologic variability in plasma glucose, hemoglobin A1c, and advanced glycation end products associated with diabetes complications. J Diabetes Sci Tech 2009;3:635–643