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 HbA1c and fructosamine in 925 patients with type 2 diabetes to calculate the G-Gap, defined as the difference between measured HbA1c and fructosamine-based predicted HbA1c. Patients were explored for retinopathy, nephropathy, peripheral neuropathy, cardiac autonomic neuropathy (n = 512), and silent myocardial ischemia.

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 ± 1.63 vs. 0.3 ± 1.30%; P < 0.0001). Because HbA1c was associated with both G-Gap (HbA1c 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 (HbA1c 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 HbA1c. 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 HbA1c, albumin levels, and confounding factors, suggesting a specific role of intracellular glycation susceptibility on kidney glomerular changes.
data have been published on G-Gap and retinopathy (7), whereas in type 2 diabet-
es 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 be-
tween G-Gap and the presence of ne-
phropathy, 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 re-
ferred to the Diabetes Department of Jean Verdier Hospital (Bondy, France) be-
tween 1994 and 2011. None had been admitted for an acute disease or had recently modified lifestyle or treatment. All of them had fructosamine, HbA1c, and creatininemia measurements. To avoid any influence on fructosamine measure-
ment (8), we excluded individuals with renal failure (creatinine clearance <60
mL/min) (9). The patients had no known hemoglobinopathy or erythrocyte disor-
der.

Nephropathy was defined as incipi-
ent nephropathy or overt macroprotein-
uريا. Incipient nephropathy was defined as urinary albumin excretion rate between
30 and 299 mg over 24 h, and macro-
proteinuria was defined as urinary pro-
tein excretion rate >300 mg over 24 h on at least two measurements. Diabetic reti-
nopathy 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: neu-
ropathic symptoms; decreased distal sen-
sation; or decreased or absent ankle
reflexes. Cardiac autonomic neuropathy
was assessed in a subgroup of 512 pa-
tients using the following three tests as recommended (10):Valsalva; deep
breathing; and lying to standing. Age
was taken into account for test interpre-
tation as previously described (11). Car-
diac 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 nor-
mal 12-lead resting ECG, and at least one
of the following additional cardiovascular
risk factors: dyslipidemia (total chole-
sterol >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 antihy-
pertensive therapy); smoking; nephropathy;
family history of premature coronary ar-
tery disease; and peripheral or carotid oc-
clusive arterial disease. As previously
reported (12–15), each patient underwent
a 201 TI 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 ab-
normal myocardial scintigraphy imaging,
i.e., defects in at least three out of 17 seg-
mental regions, or both.

Biochemical measurements

All the other measurements were per-
formed on the second day of hospitaliza-
tion, at fasting. Glucose was measured on
venous plasma by the glucose oxidase
method (colorimetry, Kone Optima; Ther-
molab System, Paris La Défense, France).

HbA1c measurement was based on a turbi-
dimetric inhibition immunoassay principle
and total hemoglobin was measured
throughout the period of inclusion (16).
Fructosamine was measured by the nitro-
blue tetrозolium colorimeteric procedure
based on the reducing ability of fructo-
samine in alkaline solution (COBAS; Roche
Diagnostics GmbH, Penzberg, Germany)
(17). The intra-assay and interassay coeffi-
cients of variation were 1.2 and 1.6%, re-
spectively.

The following measurements also
were performed: creatinine and serum
albumin (colorimetric assay, COBAS;
Roche Diagnostics); urinary albumin ex-
cretion rate (mean of two measurements,
laser immunonephelometry, BN100;
Dade-Behring); total cholesterol; HDL
cholesterol; and triglycerides (enzymatic
colorimetry, Hitachi 912; Roche Diagnos-
tic, Meylan, France). LDL cholesterol
was calculated using the Friedwald formula.

The accuracy of all the methods used was
evaluated biannually by national (exter-
nal) 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 χ² test. Logistic regression was used
for multivariate analyses based on a
model including the factors that were asso-
ciated with a diabetes complication with
a P < 0.10 in univariate analyses. Because HbA1c and G-Gap were highly
correlated, they could not be entered to-
gether in the logistic regression models.

Therefore, we matched patients with or
without each complication for HbA1c,
to evaluate the potential association of
this complication with G-Gap, independ-
ently of HbA1c. Statistical analyses were
performed using SPSS software (SPSS, Chi-
cago, IL). P < 0.05 was considered for
statistical significance.

RESULTS

Patient characteristics

The main clinical and biological charac-
teristics 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 neuropa-
thy 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 HbA1c and HbA1c pre-
dicted from fructosamine based on the
HbA1c–fructosamine regression equation
(4–6): predicted HbA1c = 0.020 × fruc-
tosamine + 2.05 (r = 0.731; P < 0.0001)
(Fig. 1). The patients then were separated
by G-Gap tertiles (Fig. 1 and Table 1). In-
creasing G-Gap tertiles were positively
associated with female sex, BMI, the
presence of dyslipidemia, HbA1c, 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).

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, HbA1c, 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 (\( r = 0.141 \); \( P < 0.0001 \)). HbA1c and G-Gap could not be considered together in multivariate analyses because HbA1c was used to determine G-Gap.

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

|                        | Total   | G-Gap tertile 1 | G-Gap tertile 2 | G-Gap tertile 3 | \( P \)  |
|------------------------|---------|-----------------|-----------------|-----------------|--------|
| **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/m²)**        | 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 (59.3)  | 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 (42.3)  | 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|
| **HbA1c (%)**          | 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.

**Figure 1—Correlation between HbA1c and fructosamine levels and repartition of the G-Gap tertiles.**
Table 2—Parameters associated with nephropathy and macroproteinuria

| Parameter                    | No nephropathy | Nephropathy | P   | No macroproteinuria | Macroproteinuria | P   |
|------------------------------|---------------|-------------|-----|---------------------|------------------|-----|
| Age (years)                  | 57.7 ± 0.0    | 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/m²)                  | 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 |
| Hyperglycemia (%)            | 388 (64.8)    | 193 (81.1)  | <0.0001 | 499 (68.1)         | 51 (89.5)        | <0.0001 |
| Dysplasia (%)                | 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 (34.4)        | <0.0001 |
| HbA1c (%)                    | 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 HbA1c to explain macroproteinuria, all the parameters but diabetes duration and HbA1c 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 HbA1c (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 HbA1c to explain nephropathy, all the parameters, including HbA1c (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 HbA1c

Because HbA1c and G-Gap are highly linked together, the association between G-Gap and nephropathy might be attributable to an association between HbA1c and nephropathy. We therefore matched for HbA1c 54 patients with macroproteinuria with 200 patients without macroproteinuria (Table 3). HbA1c 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 HbA1c based on the HbA1c/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 HbA1c and albumin-indexed HbA1c. 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 HbA1c 216 patients with nephropathy with 216 patients free of nephropathy (HbA1c 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 HbA1c levels (retinopathy HbA1c 8.7 ± 2.0% compared with no retinopathy HbA1c 8.1 ± 1.2%; P < 0.0001; and neuropathy HbA1c 8.6 ± 2.1% compared with no neuropathy HbA1c 8.1 ± 1.9%; P < 0.0001). Therefore, we also matched for HbA1c 260 patients with retinopathy and 260 without (HbA1c 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 HbA1c 190 patients with peripheral neuropathy and 190 without (HbA1c 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.
Glycation gap and macroproteinuria

This association appears to be independent of HbA1c, 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 HbA1c. Because a reanalysis of those data showed no significant influence of hemoglobin glycation index on the risk of nephropathy if HbA1c was taken into account, this association was actually assumed to reflect an association between nephropathy and HbA1c 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 HbA1c, fructosamine, and diabetes duration were included as factors in the analysis (4). In the current study, nephropathy was associated with HbA1c 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 HbA1c effect. This procedure did not affect this association. Thus, these data demonstrate a correlation between a high G-Gap and prevalent macroproteinuria, independently of HbA1c 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 HbA1c 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 HbA1c 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 HbA1c, 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 Rodriguez-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

| Parameters associated with macroproteinuria in patients matched for HbA1c | No macroproteinuria | Macroproteinuria | Univariate | Multivariate* |
|--------------------|---------------------|-----------------|------------|--------------|
| Characteristics     | n = 200             | n = 54          | P          | OR 95% CI    | P            |
| Age (years)        | 57.7 ± 9.5          | 57.3 ± 8.6      | NS         | 3.2          | 1.5–6.7      | <0.01        |
| Sex (male/female)  | 103/94              | 42/12           | <0.0001    | 1.03         | 0.99–1.08   | 0.16         |
| BMI (kg/m²)        | 31.1 ± 6.4          | 31.5 ± 6.6      | NS         | 2.9          | 1.1–7.5     | <0.05        |
| Diabetes duration  | 11.2 ± 7.4          | 13.4 ± 7.2      | 0.053      | 1.08         | 0.16–3.2    | 0.16         |
| Hypertension (%)   | 139 (69.8)          | 48 (88.9)       | <0.05      | 1.12         | 1.1–6.3     | <0.01        |
| 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      |              |              |              |
| HbA1c (%)          | 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.
adjustment of HbA1c (5). In that study, Cox regression analyses failed to show if HbA1c 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 HbA1c 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 HbA1c (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 HbA1c 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 HbA1c 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, intrapatient 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 HbA1c 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. B.C., 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 statistical 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.

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