Haemoglobin glycation may partly explain the discordance between HbA1c measurement and oral glucose tolerance test to diagnose dysglycaemia in overweight/obese subjects

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Abstract

Aim. – This study assessed whether the poor correlation between HbA1c and oral glucose tolerance test (OGTT) for dysglycaemia diagnosis may be explained by haemoglobin glycation (HbG).

Methods. – A total of 1033 consecutive overweight or obese patients with no known diabetes underwent OGTT and measurement of HbA1c to diagnose diabetes and dysglycaemia (American Diabetes Association criteria). For each OGTT result category, low, medium and high HbG was defined according to the mean HbA1c/fructosamine ratio and mean fructosamine. High HbG was defined as values greater than mean values in each OGTT category for both HbA1c/fructosamine ratio and fructosamine levels, and low HbG was defined as lower values of both. The remaining patients were considered medium HbG.

Results. – Based on OGTT and HbA1c values, 267 (25.8%) and 443 (42.8%) patients had intermediate hyperglycaemia, and 66 (6.4%) and 95 (9.2%) patients had diabetes, respectively. The results were discordant for intermediate hyperglycaemia or diabetes diagnosis in 41.7% and for diabetes diagnosis in 10.0% of the patients. The proportion of patients with HbA1c ≥ 6.5%, but without OGTT-diagnosed diabetes, was 0%, 3.8% and 32.8% in the low-HbG, medium-HbG and high-HbG groups, respectively. In contrast, the proportion of patients with HbA1c < 5.7%, but with an abnormal OGTT, was 30.4%, 11.1% and 0%, respectively. The AUROC of HbA1c to detect OGTT-diagnosed diabetes was better in the medium-HbG group [0.874 (0.816–0.931)] than in those with low or high HbG [0.628 (0.489–0.768); P < 0.01]. Only age was independently associated with high-HbG status [10-year OR: 1.3 (1.1–1.5); P < 0.0001].

Conclusion. – Haemoglobin glycation may explain many of the discordant results between HbA1c and OGTT when used for dysglycaemia diagnosis.

Keywords: Haemoglobin glycation; HbA1c; Oral glucose tolerance test; Obesity; Diabetes diagnosis; Dysglycaemia diagnosis

Résumé

La glycation de l’hémoglobine pourrait expliquer la discordance de l’HbA1c et de la charge en glucose pour diagnostiquer les dysglycémies chez les sujets en surpoids ou obèses.

Objectif. – Évaluer si la faible concordance entre HbA1c et charge orale en glucose (COG) pour le diagnostic des dysglycémies pourrait être expliquée par la glycation de l’hémoglobine (Ghb).

Méthodes. – Mille trente-trois patients consécutifs en surpoids ou obèses sans diabète connu ont eu une COG et leur HbA1c, mesuré pour diagnostiquer un diabète ou une dysglycémie (critères de l’American Diabetes Association). Pour chaque catégorie de résultat de la COG, nous avons défini une GHb faible, moyenne ou forte selon la valeur moyenne du rapport HbA1c/fructosamine et de la concentration de fructosamine.

Abbreviations: Alb-F, albumin-corrected fructosamine; AUROC, area under the receiver operating characteristic curve; BMI, body mass index; HbA1c, glycosylated haemoglobin; HbG, haemoglobin glycation; HOMA-IR, homeostasis model assessment for insulin resistance; OGTT, oral glucose tolerance test.

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Une GHb forte a été définie par des valeurs du rapport HbA1c/fructosamine et de la fructosamine plus fortes que leurs moyennes dans la catégorie de COG ; et une GHb faible par des valeurs plus faibles. Les patients restants avaient une GHb moyenne.

**Résultats.** — Selon les résultats de la COG et d’HbA1c, respectivement 267 (25,8 %) et 443 (42,8 %) patients avaient une hyperglycémie intermédiaire ; 66 (6,4 %) et 95 (9,2 %) patients avaient un diabète. Les résultats étaient discordants pour le diagnostic d’hyperglycémie intermédiaire ou de diabète chez 41,7 % des patients et pour le diagnostic de diabète chez 10,0 % d’entre eux. Les proportions de patients avec une HbA1c ≥ 6,5 % mais sans diabète diagnostiqué par la COG étaient de 0,3 et 32,8 % respectivement dans les groupes faible, moyenne et forte GHb. Réciproquement, les proportions de patients avec une HbA1c < 5,7 % mais avec une COG anormale étaient respectivement de 30,4, 11,1 et 0 %. L’AROC de l’HbA1c pour détecter un diabète défini par la COG était meilleure chez les patients avec une GHb moyenne (0,874 [0,816–0,931]) que chez les patients avec une GHb faible ou forte (0,628 [0,489–0,768]) (P < 0,01). Seul l’âge était indépendamment associé à une forte GHb (odds ratio pour dix ans 1,3 [1,1–1,5], P < 0,0001).

**Conclusion.** — La glycation de l’hémoglobine pourrait expliquer de nombreux résultats discordants entre HbA1c et la COG dans le cadre du diagnostic des dysglycémies.

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**Mots clés :** Glycation de l’hémoglobine ; HbA1c ; hyperglycémie provoquée orale ; Obésité ; Diagnostic de diabète ; diagnostic de dysglycémie

## 1. Introduction

The criteria for diabetes diagnosis are currently based on the ability of fasting plasma glucose (FPG), 2-h plasma glucose after an oral glucose tolerance test (OGTT) and glycated haemoglobin (HbA1c) to identify people at high risk of diabetic retinopathy [1–3]. Impaired fasting glucose, impaired glucose tolerance and elevated HbA1c are associated with an increased risk of developing diabetes and are components of so-called “intermediate hyperglycaemia” [1,2,4–6]. For this reason, FPG, 2-h plasma glucose and, more recently, HbA1c have been considered by the American Diabetes Association (ADA) as diagnostic criteria for diabetes and intermediate hyperglycaemia [7]. It is recommended to screen for dysglycaemia in subjects with the highest risk and especially those who are overweight or obese [8].

However, there is increasing evidence of discordant results compared with OGTT when HbA1c is used for diabetes diagnosis and even more so for diagnosing intermediate hyperglycaemia [9–11]. These discordances between HbA1c and OGTT were initially attributed to inadequate or poor measurement reliability, but have remained unchanged despite standardization procedures [12]. There are also limitations with HbA1c measurement. First, some haemoglobin traits have been reported to interfere with some assay methods despite improvements in the most recent affinity assays [13]. Second, any condition with a change in red-cell turnover such as haemolytic anaemia, major blood loss or transfusions will lead to false HbA1c results. Third, HbA1c variations related to racial disparities [14] and age [2,11,15] have been suggested, but have not led to adapted norms so far. Finally, haemoglobin glycation (HbG) may be different in each individual. For example, blood glucose concentrations were reported to account for no more than one-third of the variance of HbA1c among non-diabetic individuals [16], in whom between-subject variation in HbA1c is almost three times that of within-subject variation [17]. HbG can be evaluated by comparing the non-enzymatic glycation of haemoglobin in the intraerythrocyte compartment (HbA1c) and of proteins in the same compartment as plasma glucose (fructosamine).

The hypothesis that subjects with a high HbG would have higher HbA1c values than expected was raised when considering the results of their OGTTs and led to the idea that HbA1c ≥ 6.5% would not necessarily correspond to OGTT-diagnosed diabetes. Similarly, subjects with low HbG would have a low HbA1c, whereas the OGTT would be abnormal. The objective of the present study was therefore to test this hypothesis in an established cohort of overweight or obese patients at high risk *a priori* of dysglycaemia with fructosamine and HbA1c measurement as well as OGTT, and to investigate the factors associated with high HbG.

## 2. Patients

The cohort included 1444 adult inpatients referred to our department between January 1998 and February 2006 for management of overweight [body mass index (BMI) ≥ 25 kg/m²] or obesity (BMI ≥ 30 kg/m²) who had not been previously diagnosed with diabetes. Also, none of them had been admitted for an acute disease or had recently modified their lifestyle. From this cohort, 1227 patients who had available measurements of albumin, creatinine, thyroid-stimulating hormone (TSH), fructosamine and HbA1c, and an OGTT with FPG and 2-h plasma glucose measurement, were selected. To avoid any analytical issues with fructosamine measurement, individuals who had abnormal TSH measurements were excluded in case of altered protein turnover, as were those who had renal failure, which influences fructosamine results [18].

### 2.1. Biochemistry measurements

The OGTT was performed in the morning after a 12-h fast. The test consisted of plasma glucose measurements when fasting and 120 min after drinking 75 g of anhydrous glucose dissolved in 200 mL of water within 5 min. Patients were also asked to eat 250 g of jam the afternoon before the OGTT to compensate for any carbohydrate restriction the previous day [8]. According to ADA criteria, diabetes was defined as FPG ≥ 7 mmol/L (126 mg/dL) or a 2-h plasma glucose ≥ 11.1 mmol/L (200 mg/dL) based on the OGTT, and an HbA1c ≥ 6.5% (48 mmol/mol) based on the HbA1c [7]. Intermediate hyperglycaemia was defined as impaired fasting glucose [FPG ≥ 5.6 mmol/L (100 mg/dL) and < 7 mmol/L]
and/or impaired glucose tolerance [2-h plasma glucose ≥ 7.8 mmol/L (140 mg/dL) and < 11.1 mmol/L] with the OGTT strategy, and as HbA1c ≥ 5.7% (39 mmol/mol) and < 6.5% (48 mmol/mol) with the HbA1c strategy [7]. Dysglycaemia was defined as intermediate hyperglycaemia or diabetes. All other measurements were performed on the same day.

Glucose was measured in venous plasma by glucose-oxidase colorimetry (Kone Optima, Thermobal System, Paris La Défense, France) and HbA1c measurement was based on turbidimetric inhibition immunoassay, and total haemoglobin was measured by a modified alkaline haematin reaction using Dimension® technology (Siemens Healthcare Diagnostics, Malvern, PA, USA) as previously described [9].

Fructosamine was measured by the nitroblue tetrarzsilum colorimetric procedure based on the reducing ability of fructosamine in alkaline solution using COBAS (Roche Diagnostics GmbH, Mannheim, Germany) [19]. The intra-and inter-assay coefficients of variation were 1.2% and 1.6%, respectively. Fructosamine values were corrected for variations in concentrations of serum albumin (colorimetric assay) [20] according to the following formula by Lamb et al. [21]: Alb-F = fructosamine (μmol/L) × 100/albumin (g/L).

Thyroid-stimulating hormone (TSH) was measured by electrochemiluminescence immunoassay using COBAS (Roche Diagnostics; normal values: 0.27–4.20 μIU/L), and renal failure was defined as creatinine clearance < 60 mL/min by colorimetric assay also using COBAS (Roche Diagnostics) [22]. Serum insulin was measured using a luminescent immunoassay analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA) and a homoeostasis model assessment for insulin resistance (HOMA-IR) index was calculated. Measurements were also performed for total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides (enzymatic colorimetry, Hitachi 912 analyzer, Roche Diagnostics France, Meylan, France). Low-density lipoprotein (LDL) cholesterol was calculated using Friedewald’s formula. The accuracy of all these methods was evaluated twice a year by national (external) quality-control surveys.

2.2. Determination of patients with low or high HbG

The first consideration was the glycosylation gap, defined as the difference between measured HbA1c and the HbA1c predicted from fructosamine [18,23]. The larger the glycosylation gap, the higher the haemoglobin glycation compared with protein glycation. The glycosylation gap was calculated as the difference between measured HbA1c and HbA1c predicted from Alb-F, based on the HbA1c–Alb-F regression equation. However, the correlation between HbA1c and Alb-F was weak (r = 0.185, P < 0.0001). This may have been peculiar to the present cohort of patients with no known diabetes, as the correlation between Alb-F and HbA1c in 932 type 2 diabetic patients in our department, using the same methods and over the same period of time, was similar (r = 0.67, P < 0.001) to that found in the first published report on the glycosylation gap in diabetic patients [18,23]. Furthermore, the glycosylation gap was strongly linked to HbA1c levels. For this reason, a new index of HbG was created, based on the level of HbA1c and Alb-F in each category of glucose metabolism according to the OGTT.

The ratio between HbA1c (haemoglobin glycation) and Alb-F (other protein glycation) was calculated. An increase in this ratio may result from either high haemoglobin glycation (HbA1c greater than the mean value in each OGTT category) or low protein glycation (Alb-F lower than the mean value in each OGTT category). The patients considered with high HbG were those with a high ratio without – not due to – low Alb-F. Likewise, the patients with a low HbG were defined as those with a low ratio without high Alb-F. The remaining patients were considered to be medium HbG, although some had a high ratio most likely resulting from a low Alb-F, while others had a low ratio most likely resulting from a high Alb-F (Fig. 1). High or low values of HbA1c/Alb-F ratio and Alb-F were defined as those greater or less than their respective means in a given glucose metabolism category (according to OGTT results). Mean HbA1c/Alb-F ratios were 0.0114, 0.0117 and 0.0120% g/μmol in subjects with normal OGTT, intermediate hyperglycaemia and diabetes, respectively, according to OGTT, and the mean Alb-F was 493, 499 and 534 μmol/g in these three categories, respectively (Fig. 1).

2.3. Statistical analyses

Continuous variables were expressed as means ± D values and compared by one-way analysis of variance (ANOVA) or the Mann–Whitney U test for adequacy. 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 associated with high HbG and P value ≤ 0.01 on univariate analyses, with origin and gender forced into another model. The C statistic (area under the receiver operating characteristic curve, AUROC) was used to determine whether the performance of HbA1c in detecting OGTT-diagnosed diabetes or dysglycaemia (intermediate hyperglycaemia or diabetes) was better in the medium-HbG group than in patients with low or high HbG. Sensitivity, specificity, and positive and negative predictive values of HbA1c (ADA thresholds) to detect OGTT-diagnosed diabetes or an abnormal OGTT were also compared. Statistical analyses were carried out using SPSS software (SPSS, Chicago, IL, USA). The 0.05 probability level was considered statistically significant.

3. Results

3.1. Patients’ characteristics according to OGTT and HbA1c, and concordance of results

A total of 1227 patients all had available measurements. Of these patients, 113 had hypothyroidism (two with renal failure), 31 had hyperthyroidism (three with renal failure) and 50 further patients had renal failure. The characteristics of the 1033 patients who were ultimately included in the study are presented in Table 1.

Based on OGTT, 267 patients (25.8%) had intermediate hyperglycaemia (80 with impaired fasting glucose,
Fig. 1. Characterization of subjects with low, medium and high haemoglobin glycation (HbG) according to HbA1c/albumin-corrected fructosamine (Alb-F) ratio for each oral glucose tolerance test (OGTT) category.

Table 1
Characteristics of the overall cohort and subgroups according to haemoglobin glycation (HbG).

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 1033)</th>
<th>Low HbG (n = 135)</th>
<th>Medium HbG (n = 782)</th>
<th>High HbG (n = 116)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometry and demographics</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>40.1 ± 13.0</td>
<td>38.6 ± 11.6</td>
<td>39.6 ± 13.0</td>
<td>45.1 ± 13.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender, male/female</td>
<td>173/860</td>
<td>19/116</td>
<td>135/647</td>
<td>19/97</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>37.1 ± 6.9</td>
<td>36.3 ± 6.3</td>
<td>37.1 ± 6.8</td>
<td>38.3 ± 7.6</td>
<td>0.072</td>
</tr>
<tr>
<td>Family history of diabetes, %</td>
<td>407 (39.4)</td>
<td>47 (34.8)</td>
<td>314 (40.2)</td>
<td>46 (39.7)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Origin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europe, %</td>
<td>775 (75.2)</td>
<td>109 (80.7)</td>
<td>577 (74.0)</td>
<td>89 (76.7)</td>
<td>NS</td>
</tr>
<tr>
<td>North Africa, %</td>
<td>143 (13.9)</td>
<td>18 (13.3)</td>
<td>112 (14.4)</td>
<td>13 (11.2)</td>
<td></td>
</tr>
<tr>
<td>Africa, %</td>
<td>77 (7.5)</td>
<td>4 (3.0)</td>
<td>63 (8.1)</td>
<td>10 (8.6)</td>
<td></td>
</tr>
<tr>
<td>Asia, %</td>
<td>36 (3.5)</td>
<td>4 (3.0)</td>
<td>28 (3.6)</td>
<td>4 (3.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Cardiovascular risk factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertensiona, %</td>
<td>325 (32.8)</td>
<td>41 (32.3)</td>
<td>243 (32.2)</td>
<td>41 (36.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Dyslipidaemib, %</td>
<td>338 (37.1)</td>
<td>44 (39.3)</td>
<td>253 (36.6)</td>
<td>41 (38.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.1 ± 1.0</td>
<td>5.2 ± 1.1</td>
<td>5.1 ± 1.0</td>
<td>5.1 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.4 ± 1.0</td>
<td>1.4 ± 0.9</td>
<td>1.4 ± 1.1</td>
<td>1.3 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.3 ± 0.4</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.4</td>
<td>1.3 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.2 ± 0.9</td>
<td>3.3 ± 1.0</td>
<td>3.2 ± 0.9</td>
<td>3.1 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking habits, %</td>
<td>207 (20.9)</td>
<td>30 (22.7)</td>
<td>158 (21.2)</td>
<td>19 (16.7)</td>
<td>NS</td>
</tr>
<tr>
<td>The metabolic syndromec, %</td>
<td>426 (42.8)</td>
<td>49 (40.2)</td>
<td>320 (41.9)</td>
<td>57 (51.8)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Glycaemic/metabolic status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/L</td>
<td>4.9 ± 0.8</td>
<td>4.8 ± 0.7</td>
<td>4.9 ± 0.8</td>
<td>5.1 ± 0.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2-h plasma glucose, mmol/L</td>
<td>6.7 ± 2.3</td>
<td>7.0 ± 2.5</td>
<td>6.7 ± 2.2</td>
<td>7.0 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.7 ± 0.6</td>
<td>5.0 ± 0.4</td>
<td>5.7 ± 0.5</td>
<td>6.4 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fructosamine, µmol/L</td>
<td>210 ± 21</td>
<td>206 ± 16</td>
<td>210 ± 22</td>
<td>218 ± 18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>42.5 ± 3.4</td>
<td>43.3 ± 3.1</td>
<td>42.4 ± 3.5</td>
<td>42.1 ± 3.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HbA1c/Alb-F ratio, %/µmol</td>
<td>0.115 ± 0.001</td>
<td>0.106 ± 0.001</td>
<td>0.115 ± 0.002</td>
<td>0.123 ± 0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.0 ± 2.2</td>
<td>2.9 ± 2.1</td>
<td>3.0 ± 2.3</td>
<td>3.2 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Alb-F, µmol/g</td>
<td>497.1 ± 50.9</td>
<td>475.1 ± 22.3</td>
<td>497.6 ± 55.9</td>
<td>519.1 ± 20.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abnormal OGTT, %</td>
<td>333 (32.2)</td>
<td>48 (35.6)</td>
<td>243 (31.1)</td>
<td>42 (36.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes according to OGTT, %</td>
<td>66 (6.4)</td>
<td>12 (8.9)</td>
<td>47 (6.0)</td>
<td>7 (6.0)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD or n (%). NS: not significant; HOMA-IR: homoeostasis model assessment for insulin resistance; Alb-F: albumin-corrected fructosamine; OGTT: oral glucose tolerance test.

a Blood pressure ≥ 140/90 mmHg or treatment for hypertension.
b HDL cholesterol < 0.9 mmol/L (35 mg/L) and/or triglycerides > 2.82 mmol/L (250 mg/L) and/or treatment for dyslipidaemia.
c As defined by the International Diabetes Federation.
Table S1; see supplementary material associated with this article online). In addition, the specificity and positive predictive value of HbA1c ≥ 6.5% to detect OGTT-diagnosed diabetes were better in patients with medium HbG than in those with either low or high HbG, and the sensitivity and negative predictive value of HbA1c ≥ 5.7% to detect an abnormal OGTT were better in patients with medium HbG than in those with either low or high HbG (Table S1).

### 3.3. Parameters associated with high haemoglobin glycation

An increased HbG was, as expected, associated with HbA1c, Alb-F, fructosamine, albumin and HbA1c/Alb-F ratio as well as with age and FPG, with a trend for BMI (P=0.072; Table 1).

Multivariate analysis including age, BMI and FPG found that only age [10-year odds ratio (OR): 1.3, 95% CI: 1.1–1.5; P<0.001] was independently associated with high HbG. The result was similar when gender and origin were added and forced into the model. The effect of age can be demonstrated by the following example: in patients without diabetes according to OGTT, the prevalence of high HbG was 6.6%, 8.4%, 13.6% and 17.1% in patients aged <30, 30–39, 40–49 and ≥ 50 years, respectively, with a prevalence of HbA1c ≥ 6.5% of 3.3%, 5.0%, 9.9% and 9.5%.

### 4. Discussion

HbA1c measurement has recently been proposed as a diagnostic tool for diabetes and intermediate hyperglycaemia. The present cohort of patients who were overweight or obese confirms that the concordance of the HbA1c and OGTT strategies is poor for diabetes diagnosis [9,11] and even worse for dysglycaemia classification [9,10]. Indeed, inconsistent results were found for diabetes and dysglycaemia diagnosis in 10.0% and 41.7%, respectively, of our study population. This suggests that if OGTT and HbA1c measurement identify people with the same risk of incident diabetes and prevalent retinopathy, they are not diagnosing the same patients. In the 1990s, the correlation between HbA1c and fasting or 2-h plasma glucose levels was reported to be much lower in subjects with no known diabetes than in diabetics [16]. However, it is assumed that the wide assay variability in HbA1c measurement at the time was making...
Fig. 2. Concordance of HbA1c measurement and oral glucose tolerance test (OGTT) results in the diagnosis of diabetes (A) and of intermediate hyperglycaemia or diabetes (dysglycaemia) (B) according to haemoglobin glycation (HbG).

a larger contribution to the range of HbA1c in non-diabetic individuals than in diabetic patients. Our present study shows for the first time that, in fact, haemoglobin glycation may be involved in these discrepancies: 32.8% of patients with high HbG had an HbA1c ≥ 6.5% whereas OGTT did not diagnose diabetes. Likewise, 30.4% of those with low HbG had an HbA1c < 5.7% whereas their OGTT was abnormal. HbG appears to be associated with increasing age. The implication of HbG in the poor concordance of HbA1c and OGTT was further demonstrated by the C statistic showing that the AUROC of HbA1c to detect OGTT-diagnosed diabetes was higher when considering only those with medium HbG. Furthermore, the performance of the HbA1c thresholds to detect OGTT abnormalities was also better in patients with medium HbG than in those with either low or high HbG.

Our present study has used a novel index of HbG because the most well-known one – the glycosylation gap – is apparently not accurate in subjects without known diabetes. In addition, the larger the glycosylation gap, the higher the HbA1c level. Therefore, it was difficult to determine whether changes in the glycosylation gap itself or in HbA1c were responsible for any abnormality. Use of the HbA1c level controlled for 2-h glucose was an alternative [16], but FPG values were not taken into account. Our present study addressed these issues by considering both the HbA1c/Alb-F ratio and Alb-F for each OGTT category to define HbG.

Several mechanisms may be involved in the differential glycation of haemoglobin and other proteins, and a heritable component has been demonstrated for the glycosylation gap [24]. The lifespan of erythrocytes should be taken into account, but our HbA1c measurement was not sensitive to haemoglobin traits [9]. Interindividual differences in the turnover/metabolism of underlying proteins should also be discussed when considering fructosamine measurement. However, patients without renal failure or abnormal thyroid function were selected [18], and albumin-corrected fructosamine values (Alb-F) were considered [20,21]. Variation in haemoglobin and protein glycation can also be the result of different glucose concentrations in the intracellular and extracellular compartments, and was suggested by greater glucose concentrations at a given average plasma glucose concentration in the intraerythrocyte compartment than in plasma in subjects with the highest HbA1c levels controlled for 2-h glucose [25]. This mechanism, however, is unlikely to be strong enough to affect the glycation process. Khera et al. [26] have shown that, at steady state, there is a sugar concentration gradient across the human erythrocyte membrane that varies between individuals, and that this gradient is correlated with HbA1c and the glycosylation gap, but not fructosamine level. The
intraerythrocyte environment may also be involved. For example, levels of erythrocyte 2,3-diphosphoglycerate, a catalyst of glycation, have been reported to be elevated in subjects with higher-than-expected HbA1c levels controlled for 2-h glucose [25]. Furthermore, the patients included in the present study were hospitalized for the first time and had not changed their lifestyles during the previous weeks. Differences in the duration of exposure to high glucose levels may therefore minimally account for the discrepancy between haemoglobin and protein glycation.

Yudkin et al. [16], looking at the mean 2-h plasma glucose and mean HbA1c in 223 subjects without known diabetes, could find no determinant of haemoglobin glycation among age, gender, BMI or haemoglobin level. Interestingly, in our present population of 1033 overweight or obese patients, high HbG was associated with age and was independent of other parameters. Age has previously been reported as a factor explaining variations of HbA1c levels [2,11,15]. As HbG increases with ageing, an HbA1c ≥ 6.5%, which is not very high, should perhaps be used with caution to define diabetes, and especially in older subjects.

There were some limitations to our study. The diagnosis of diabetes or intermediate hyperglycaemia was based on a single OGTT and HbA1c measurement, and the 1-h plasma glucose, shown to predict incident type 2 diabetes, was not measured [27]. HbG was also not investigated either in vivo or in vitro. The patients who were included had a BMI > 25 kg/m², so our present results are not necessarily generalizable to the population with normal body weight. However, evidence-based guidelines for type 2 diabetes prevention clearly recommend the detection of dysglycaemia in at-risk individuals [8] and particularly in those with overweight or obesity. More important, however, BMI was found here to not be associated with HbG. In addition, it was not possible to evaluate the influence of HbG on the concordance of HbA1c and OGTT by ethnic group as our study population included only a small number of non-European patients.

In conclusion, OGTT and HbA1c screening strategies for intermediate hyperglycaemia and diabetes can identify individuals with the same risks, but do not identify the same patients. Our present study suggests that, at least in overweight and obese patients with a small range of glucose abnormalities, HbG may partly account for such discrepancies. A prospective study evaluating the risk of developing diabetes according to HbA1c levels and HbG, and an epidemiological study evaluating the association between HbA1c and HbG and prevalent retinopathy, are also mandatory to confirm these data.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgments

E.C. directed the research, performed the statistics and wrote the manuscript; S.C., C.C.P. and I.B. contributed to the discussion; E.H.T. researched data; M.T.N. researched data and worked on statistics; M.A. worked on statistics; N.C. contributed to the discussion, reviewed/edited the manuscript; and P.V. directed the research, contributed to the discussion and reviewed/edited the manuscript.

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Appendix. Supplementary data

Supplementary data (Table S1) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.diabet.2012.08.013.

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