Original article

Hyperglycaemia per se does not affect erythrocyte glucose-6-phosphate dehydrogenase activity in ketosis-prone diabetes

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Abstract

Aim. – Previously, we described patients with ketosis-prone type 2 diabetes (KPD) and glucose-6-phosphate dehydrogenase (G6PD) deficiency, but no mutation of the G6PD gene. Our present study used two complementary approaches to test whether hyperglycaemia might inhibit G6PD activity: (1) effect of acute hyperglycaemia induced by glucose ramping; and (2) effect of chronic hyperglycaemia using correlation between G6PD activity and HbA1c levels.

Methods. – In the first substudy, 16 KPD patients were compared with 11 healthy, non-diabetic control subjects of the same geographical background. Erythrocyte G6PD activity and plasma glucose were assessed at baseline and every 40 min during intravenous glucose ramping that allowed maintaining hyperglycaemia for more than 3 h. In the second substudy, erythrocyte G6PD activity and HbA1c levels were evaluated in 108 consecutive African patients with either type 2 diabetes or KPD, and a potential correlation sought between the two variables.

Results. – The maximum plasma glucose level after 200 min of glucose perfusion was 20.9 ± 3.7 mmol/L for patients and 10.7 ± 2.3 mmol/L for controls. There was no difference between baseline and repeated G6PD activity levels during acute hyperglycaemia in either KPD patients (P = 0.94) or controls (P = 0.57), nor was there any significant correlation between residual erythrocyte G6PD activity and HbA1c levels (r = −0.085, P = 0.38).

Conclusion. – Neither acute nor chronic hyperglycaemia affects erythrocyte G6PD activity. Thus, hyperglycaemia alone does not explain cases of G6PD deficiency in the absence of gene mutation as described earlier.

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1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is an intracellular enzyme that catalyzes the reduction of nicotinamide adenine dinucleotide phosphate (NADP) to form NADPH, the principal agent that protects against oxidative stress in many human cell types [1]. G6PD deficiency is an X-linked genetic disorder responsible for favism (acute haemolytic anaemia), and
is highly prevalent in sub-Saharan Africans (SSAs) [1]. Some reports have suggested a link between G6PD deficiency and abnormal glucose regulation. Indeed, subjects with favism are reported to have a higher frequency of abnormal glucose tolerance [2]. Also, lower glucose-stimulated insulin secretion was described in G6PD-deficient men compared with control subjects [3]. Over the past two decades, an atypical form of diabetes called ‘ketosis-prone type 2 diabetes’ (KPD) has been widely described in people of SSA origin and shown to share numerous metabolic similarities with classical type 2 diabetes [4–6]. Ketotic onset, one of its hallmarks, is due to a transient deficiency in insulin secretion [7,8]. This might be caused by glycolytic-induced oxidative stress as described earlier [9].

In an attempt to understand the mechanism behind the impairment of insulin secretion during the acute ketogenic episode in KPD, the role of increased oxidative stress through deficiency in G6PD was assessed [10]. A higher prevalence (42.3%) of G6PD deficiency was found in patients with KPD compared with non-diabetic and type 2 diabetic groups (16.9%), and a positive correlation between insulin secretion and residual erythrocyte G6PD activity was reported [10]. However, common mutations of the G6PD gene were absent in almost half the G6PD-deficient KPD patients. This prompted various hypotheses to explain the relationship between G6PD and KPD:

- hyperglycaemia with ketosis could cause G6PD deficiency;
- G6PD deficiency and KPD could be manifestations of a syndrome yet to be described;
- and a factor regulating expression of the G6PD gene could play a role.

To explore the third hypothesis, we recently tested the role of the Arg585Gln polymorphism in the gene that encodes for sterol regulatory element-binding protein (SREBP)-1, a transcription factor that stimulates G6PD gene expression, but found no association [11]. We therefore hypothesized that hyperglycaemia, through an inhibitory effect on G6PD activity, might explain those cases of G6PD deficiency in the absence of gene mutations. Indeed, hyperglycaemia has been shown to inhibit G6PD activity in β cells in vitro [12]. In the present report, two approaches were used to assess the impact of hyperglycaemia on G6PD activity in SSAs. The first investigated the effect of intravenous (IV) glucose ramp-induced hyperglycaemia in KPD patients compared with healthy control subjects. The second examined the correlation between HbA1c levels and residual erythrocyte G6PD activity (REA) in a group of SSA diabetic subjects with a wide range of G6PD activity.

2. Patients and methods

2.1. Study design

The first substudy took place at the clinical investigation centre of Saint-Louis University Hospital in Paris, France, and evaluated 16 consecutive subjects of SSA origin with KPD who were in insulin-free near-normoglycaemic remission for at least 3 months. As antidiabetic treatment, most patients were taking either metformin alone (n = 8) or combined with a sulphonylurea (n = 2); two patients were taking a sulphonylurea alone, two were using diet alone, one was taking a glinide and one was taking acarbose. These drugs were stopped at least five days before the procedures. The above patients were compared with 11 healthy controls of the same geographical origin but with normal glucose tolerance. The non-diabetic controls were recruited by advertisements and matched to the patients by geographical origin, age and gender; they had no known family history of type 2 diabetes in their first-degree relatives. Insulin-free remission in KPD was defined as maintenance of an HbA1c level ≤ 7.0% for at least 3 months after the withdrawal of insulin treatment initiated at either diabetes onset or relapse. Participants were eligible if they had normal liver, cardiovascular, pulmonary and kidney function as assessed by medical history, physical examination and routine laboratory tests. The second substudy measured G6PD activity and HbA1c in 108 consecutive SSAs with KPD or classical type 2 diabetes.

The study was approved by the Paris Saint-Louis ethics committee, and all participants gave their written informed consent to participate.

2.2. Graded glucose infusion

For the first substudy, the glucose ramp was performed after a 12-h in-hospital overnight fast. The test consisted of five consecutive 40-min intravenous infusions of 20% glucose at 2, 4, 6, 8 and 10 mg/kg body weight/min, as per a previously described procedure [13]. Arterialized blood samples, obtained by heating the participant’s hand with a warm cuff, were collected at −10 and 0 min before, and every 40 min during, the whole procedure (200 min, or T200) for determination of plasma glucose and erythrocyte G6PD activity as well as plasma insulin levels.

2.3. Measurement of erythrocyte G6PD activity and HbA1c

All G6PD assays were run in duplicate. G6PD activity was measured as previously described, using the spectrophotometric method [10]. In brief, erythrocyte homogenate was saturated with glucose-6-phosphate and NADP+, and enzyme activity determined by measurement of the rate of absorbance change at 340 nm due to the reduction of NADP+ (G6PDH reagents; Randox Laboratories Limited, Crumlin, County Antrim, UK). Total erythrocyte enzyme activity was determined in units per gram of haemoglobin (U/g Hb). The normal range was 7–29 U/g Hb. Performance characteristics of the method were evaluated at 37 °C: linearity was verified up to 30 U/g Hb; minimum detectable concentration was 1 U/g Hb. Intra-assay precision was 4.2% for low levels and 4.6% for high levels, while inter-assay precision was 6.5% for low levels and 5.1% for high levels.

Automated glucose measurement was obtained with the Roche Diagnostics Modular® PP analyzer, using the hexokinase method. Erythrocyte HbA1c measurement was measured

by immunoturbidimetry (Tina-quant method), also using the Modular PP system.

2.4. Statistical analysis

Statistical analyses were performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). The Chi² test was used to compare categorical variables, with non-parametric tests (Mann–Whitney U and Friedman tests) for quantitative variables. The Spearman correlation was used to correlate REA (residual erythrocyte G6PD activity) and HbA1c levels.

3. Results

3.1. Participants’ clinical and biochemical characteristics

The clinical and biochemical characteristics of the participants in both our substudies are shown in Table 1. Baseline plasma glucose levels were higher in KPD patients compared with non-diabetic controls. Despite receiving the maximum tolerated dose of glucose infusion, control subjects failed to achieve the target 20 mmol/L glucose level during the ramp (Fig. 1). The maximum plasma glucose level at T200 was 10.7 ± 2.3 mmol/L for controls vs 20.9 ± 3.7 mmol/L for patients (P < 0.001).

In the first substudy, insulin levels were higher in patients at baseline, but during glucose infusion, levels were not significantly different between the two groups. Baseline G6PD activity was 24.9 ± 12.0 U/g Hb in KPD patients and 21.9 ± 15.9 U/g Hb in the controls (P = 0.27). Two patients and four control subjects had G6PD deficiency.

In the second substudy, there was a male predominance in the KPD group compared with the type 2 diabetes group. On average, both type 2 diabetes and KPD patients had high HbA1c levels (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Substudy 1</th>
<th>KPD</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>16</td>
<td>11</td>
<td>–</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>1/15</td>
<td>1/10</td>
<td>0.78</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.9 ± 8.4</td>
<td>43.6 ± 7.8</td>
<td>0.72</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.4 ± 4.3</td>
<td>25.2 ± 3.1</td>
<td>0.056*</td>
</tr>
<tr>
<td>Baseline plasma glucose (mmol/L)</td>
<td>6.7 ± 1.3</td>
<td>4.9 ± 0.4</td>
<td>&lt;0.001¹</td>
</tr>
<tr>
<td>T200 plasma glucose (mmol/L)</td>
<td>20.9 ± 3.7</td>
<td>10.7 ± 2.3</td>
<td>&lt;0.001¹</td>
</tr>
<tr>
<td>Baseline plasma insulin (µU/mL)</td>
<td>10.0 ± 7.7</td>
<td>4.8 ± 2.8</td>
<td>0.044*</td>
</tr>
<tr>
<td>T200 plasma insulin (µU/mL)</td>
<td>63.3 ± 68.2</td>
<td>133.8 ± 163.3</td>
<td>0.39</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.2 ± 0.7</td>
<td>–</td>
<td>–</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Substudy 2</th>
<th>KPD</th>
<th>T2D</th>
<th>P</th>
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<tr>
<td>n</td>
<td>55</td>
<td>53</td>
<td>–</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>5/50</td>
<td>23/30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.4 ± 10.7</td>
<td>48.8 ± 12.1</td>
<td>0.12</td>
</tr>
<tr>
<td>Mean residual G6PD activity (%)</td>
<td>157 ± 71</td>
<td>160 ± 50</td>
<td>0.88*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>10.0 ± 3.1</td>
<td>11.3 ± 3.5</td>
<td>0.038*</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD or ratios; KPD: ketosis-prone type 2 diabetes; T2D: type 2 diabetes.

* Mann–Whitney U test.

3.2. Effects of hyperglycaemia on G6PD activity

The profile of G6PD activity during glucose infusion (Fig. 2) showed no differences between the baseline and repeated G6PD activity levels during hyperglycaemia in neither the patients (P = 0.94) nor controls (P = 0.57). Also, neither patients with G6PD deficiency nor those with normal G6PD activity showed any significant changes in G6PD activity over the course of glucose ramping. As depicted in Fig. 3, the correlation between REA and HbA1c levels was not significant (r = -0.085, P = 0.38). The Spearman correlation coefficient was -0.11 (P = 0.44) in the type 2 diabetes subgroup and -0.067 (P = 0.62) in the KPD subgroup.

4. Discussion

In the present report, erythrocyte G6PD activity was not inhibited by acute hyperglycaemia sustained for at least 3 h in healthy individuals of SSA origin as well as in those with well-controlled KPD. It was also observed that, in diabetic patients, erythrocyte G6PD activity did not correlate with HbA1c, the main marker of chronic hyperglycaemia and thus an indicator of glucotoxicity. Earlier, we had proposed that KPD might be a subtype of type 2 diabetes, as it shares many of its metabolic characteristics [5]. However, a precipitating factor
such as glucotoxicity-induced oxidative stress triggers the acute secretory dysfunction [9]. As G6PD is a major reductant in cells, the role of its deficiency in the pathogenesis of KPD in Africans was strongly suggested by recent reports. Two case reports described the coincidence of a KPD-revealing ketotic [14] or keto-acidotic [15] onset and haemolysis in patients who were subsequently found to have G6PD deficiency. Whether this might be due to a potential link between ketosis and haemolysis is not known.

In our previous study assessing the potential role of G6PD deficiency in KPD, despite the higher frequency of G6PD deficiency in KPD patients compared with type 2 diabetic patients and healthy control subjects, 48% of the G6PD-deficient patients displayed none of the G6PD gene mutations commonly found in Africans [10]. This raised (1) the issue of a potential role of a transcription factor regulating the G6PD gene, and (2) the question of whether G6PD deficiency might be secondary to hyperglycaemia in some patients as part of the glucotoxicity process. The first hypothesis was tested by assessing the role of an ethnic-specific polymorphism (Arg585Gln) in the gene for SREBP-1, a transcription factor known to regulate the G6PD gene, among other activities [11]; however, no association was found between the polymorphism and G6PD activity.

The present study assessed the second question, and our findings suggest neutral effects for both acute and chronic hyperglycaemia on G6PD activity. Two experimental studies have previously investigated the role of hyperglycaemia on G6PD activity. Xu et al. [16] found that chronic hyperglycaemia decreased G6PD activity in diabetic rat kidney cortex cells, and the decrease was normalized by glucose control after insulin treatment. Zhang et al. [12] showed that β-cell G6PD expression was inhibited in vitro by 72-h exposure to high glucose levels in cultured human and mouse islets. Both studies support an inhibitory role of hyperglycaemia on G6PD activity. However, our present study failed to show similar results for erythrocyte G6PD activity in humans.

As kidney and β cells are nucleated cells, whereas erythrocytes are not, the difference between our results and findings in kidney and β cells raises the hypothesis that hyperglycaemia might inhibit G6PD activity in nucleated cells, but not in cells devoid of nuclei, owing to a transcriptional effect. Indeed, in the above-mentioned report [12], there was a parallel decrease in G6PD protein expression caused by hyperglycaemia. Another potential relationship between hyperglycaemia and G6PD is that acute hyperglycaemia might suppress G6PD activity if associated with glucotoxicity. This is especially of interest as our previous report [10] was based on patients evaluated a few days after the acute ketotic phase whereas, in our present first substudy, acute hyperglycaemia was transiently induced in well-controlled KPD patients. Nevertheless, the possibility that ketosis may have an impact on G6PD activity cannot be ruled out and deserves further study. In our second substudy, although patients had elevated HbA1c that may have indicated pre-existing glucotoxicity, not all of them were in an acute state.

In conclusion, our present study suggests that neither acutely induced nor chronic hyperglycaemia inhibits erythrocyte G6PD activity. Thus, hyperglycaemia per se does not explain G6PD deficiency in the absence of gene mutations in KPD patients, as described earlier.

Disclosure of interest

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

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Appendix A. Supplementary data

Supplementary data (French abstract) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.diabet.2014.07.002.

References