Indices of beta-cell function: association with diabetes control in patients with type 2 diabetes on stable GLP-1 agonist treatment

Preeshila Behary¹
MRCP

Ian F Godsland²
PhD, BA

Kevin C Baynes¹
FRCP, PhD

¹Diabetes and Endocrinology, Ealing Hospital NHS Trust, UK
²Endocrinology and Medicine, Department of Medicine, Imperial College London, UK

Correspondence to:
Kevin Baynes, FRCP, PhD, Ealing Hospital NHS Trust, Uxbridge Rd, Southall, Middlesex UB1 3HW, UK; email: kevin.baynes@nhs.net

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Abstract
The aims of this study were to investigate the association between metabolic factors and glycaemic control in patients with type 2 diabetes on glucagon-like peptide-1 (GLP-1) agonists, and explore the physiological reasons for less good glucose control in some patients compared to others.

We conducted a cross-sectional study of 26 patients with type 2 diabetes and HbA1c ranging from 5.7–11% (39–97 mmol/mol), on GLP-1 agonists with oral hypoglycaemics for at least four months. A liquid meal tolerance test was performed and samples for plasma glucose, insulin and C-peptide were taken at 0, 30 and 120 minutes. Glucagon was measured in the fasting state. Insulin secretion and resistance indices were correlated with HbA1c using Pearson correlation coefficient.

We found a significant correlation between HbA1c and indices of beta-cell reserve (HOMA B: r = -0.53, p<0.05; Insulinogenic 30 Index: r = -0.46, p<0.05; Disposition Index 30: r = -0.53, p<0.05; and 120 minute C-peptide: r = -0.4, p<0.05). We did not observe any significant correlation between glycaemic control and fasting glucagon nor with HOMA IR.

Indices of beta-cell function, but neither insulin sensitivity nor fasting glucagon, are associated with diabetes control in long-term GLP-1 treated patients with type 2 diabetes. This has important implications for the management of individuals who fail to achieve target glycaemic control on GLP-1 agonists. Copyright © 2014 John Wiley & Sons.

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Key words
GLP-1 agonists; type 2 diabetes

Introduction
As therapeutic agents for type 2 diabetes mellitus, glucagon-like peptide-1 (GLP-1) agonists have been developed to target a number of the pathophysiologic defects of the disease spectrum. These metabolic abnormalities include impaired insulin release in response to a glucose load, insulin resistance, increased glucagon release despite hyperglycaemia and perhaps impaired release of incretins.¹

In subjects with normoglycaemia, insulin secretion in response to a glucose load is biphasic. The first phase provides a transient high amplitude burst of insulin release whereas the second phase is of lower amplitude, but is sustained until blood glucose levels fall to baseline.

Impairment of the first phase response is generally the first sign of declining beta-cell function.³ Nevertheless, appreciable beta-cell reserve may remain after the first phase response has been entirely lost⁴ and there has to be substantial net loss of beta-cell function for diabetes to manifest; for example, in the UK Prospective Diabetes Study (UKPDS), based on homeostasis model assessment of beta-cell function, it was estimated that at diagnosis patients with type 2 diabetes had lost approximately 50% of their beta-cell function.³

At the same time, alpha-cell dysfunction and hyperglucagonaemia also contribute to the maintenance of the hyperglycaemic state. Glucagon levels are inappropriately elevated in individuals with type 2 diabetes – in both the fasting and postprandial state – contributing to increased hepatic glucose output and hence hyperglycaemia.⁵,⁷

Endogenous GLP-1, produced by intestinal L cells, stimulates insulin secretion in a glucose-dependent fashion and inhibits glucagon secretion.⁸ The GLP-1 receptor agonist drugs were developed as dipeptidyl peptidase-4 (DPP4) resistant compounds with half-lives much longer than native GLP-1.⁹ Clinical studies have shown improved beta-cell function, including partial restoration of...
first phase and second phase insulin secretion, with both liraglutide and exenatide.  

Inhibition of glucagon secretion, in both fasting and postprandial states, has also been documented with liraglutide and exenatide. 

In clinical practice not all patients treated with these drugs achieve ideal glucose control despite good concordance. For instance, even in clinical trials combining two oral hypoglycaemic drugs with a GLP-1 agonist more than 40% remain uncontrolled.  

We designed this study to explore the physiological reasons for less good glucose control in patients with type 2 diabetes on stable treatment with GLP-1 agonists.

Methods

Subjects and design

This was a cross-sectional study of 26 patients with type 2 diabetes on stable doses of GLP-1 agonist injection and oral hypoglycaemic drugs, but with a range of glucose control. Inclusion criteria included HbA1c <11% (97mmol/mol), using GLP-1 agonist therapy for at least three months with no change in oral hypoglycaemic agents. Exclusion criteria included current use of insulin, problem drinking or current use of orlistat. The study protocol was approved by the National Research Ethics Service of the UK and was conducted according to the principles of the Helsinki Declaration.

Patients taking liraglutide were asked to take their injection at 10pm for three days prior to the study. Patients using exenatide twice daily took their injection as usual. Subjects were asked not to take any strenuous exercise for 24 hours before the study. All subjects fasted for at least 8 hours.

Liquid meal tolerance test

Subjects were studied in an ambulatory care ward. An indwelling venous cannula was inserted into a forearm vein and used for venous blood sampling. Pulse and blood pressure were taken regularly as well as capillary blood glucose using a commercial meter (Accuchek).  

A liquid meal tolerance test (LMTT) was given at a dose of 3ml/kg body weight using Ensure Plus (Abbott Nutrition; 240ml provides 360kcal, 48.5g carbohydrates of which 16.5g are sugars), 15g protein and 11.8g fat).

Venous blood samples were collected for plasma glucose, insulin and C-peptide at -5, 0, 15, 30 and 120 minutes. Fasting glucagon and an HbA1c sample were also taken at -5 minutes. Blood samples for glucose and glucagon were placed on ice immediately and centrifuged within 20 minutes. Samples for insulin and C-peptide were allowed to clot at room temperature before separation. Aliquoted samples were kept frozen until analysis.

Glucose was measured by the glucose oxidase method. Insulin and C-peptide were measured using an electrochemiluminescence immunoassay with a lower detection limit for insulin of 1.39nmol/L and 0.003nmol/L for C-peptide (Roche Diagnostics, Mannheim). Glucagon was measured by an established immunoassay at our regional endocrine laboratory service with a lower limit of detection of 5pmol/L. HbA1c was measured using an automated HPLC method in a single laboratory, part of the National External Quality Assurance Scheme (UK NEQAS).

Responders and non-responders

HbA1c values prior to treatment with GLP-1 agonists were available for all 26 subjects. Those subjects who had a fall in HbA1c of ≥1% were defined as GLP-1 ‘responders’ and the rest as ‘non-responders’.

A range of indices of beta-cell function and insulin sensitivity – or its inverse, insulin resistance – were calculated as follows.

Beta-cell function in the fasting state was calculated using the homeostasis model assessment (HOMA) formula: HOMA B = 20 × Io/(Go - 3.5) [where Io = mean fasting insulin, Go = mean fasting glucose], as validated using the hyperglycaemic clamp as reference. 

An index of the phase 1 insulin response to the LMTT was provided by the Insulinogenic Index: Ingenix 30 = (Io - Io)/ (G30 - Go) [where Go = insulin at 30 minutes post commencement of the LMTT and G30 = glucose at 30 minutes post commencement of the LMTT]. The Insulinogenic Index is a widely used index in the context of the oral glucose tolerance test and has recently been adopted for use with the LMTT. An index of the phase 2 insulin response to the LMTT was provided by the plasma C-peptide concentration 120 minutes after commencement of the LMTT.

An index of beta-cell function in response to the LMTT was provided by the LMTT Disposition Index: Mtdi = Matsuda × Ingenix, which has been validated using measures derived from the intravenous glucose tolerance test (IVGTT).

An index of relative insulin resistance, derived from fasting plasma glucose and insulin concentrations was calculated using the HOMA formula: HOMA IR = (Io × Go)/22.5, as validated using the euglycaemic hyperinsulinaemic clamp.

An index of insulin sensitivity derived from LMTT plasma glucose and insulin concentrations was provided by the Matsuda Index: Matsi = 10000/((G0 × Io × Gm × Im)) [where

<table>
<thead>
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<th>Variable</th>
<th>All (n=26)</th>
<th>≥1% (n=18)</th>
<th>&lt;1% (n=8)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55±11.4</td>
<td>52.8±2.8</td>
<td>62.0±2.8</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
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<td>7.8±0.3</td>
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<td>BMI (kg/m²)</td>
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<td>NS</td>
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<tr>
<td>Duration of diabetes (years)</td>
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<td>10.9±1.2</td>
<td>12.1±2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of GLP-1 agonists (months)</td>
<td>19.9±12.2</td>
<td>18.8±2.6</td>
<td>20.5±3.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Applies to cohort achieving HbA1c reduction of ≥1% and cohort who did not.

Table 1. Characteristics of all study subjects, and subjects categorised by HbA1c reduction of ≥1% on GLP-1 analogues. (Mean ± SEM)
Glucose and fasting glucagon; 23% had a suppressed fasting glucagon detected during the study. There was no correlation between fasting glucose <4 mmol/L (72 mg/dl) and BMI. There was no correlation between fasting glucagon and HbA1c (r = -0.51, p<0.05).

Insulin sensitivity: There was no correlation between glycemic control, as assessed by HbA1c, and the indices of insulin resistance or sensitivity: HOMA IR (r = 0.373, p=NS) and Matsuda Index (r = -0.07, p=NS). HOMA IR was, nevertheless, correlated with BMI (r = 0.57, p<0.01).

Glucagon: There was no correlation between fasting glucagon and HbA1c (r = 0.15, p=NS).

None of the subjects reported nocturnal hypoglycaemia prior to the LMTT. No episode of hypoglycaemia (blood glucose <4 mmol/L [72 mg/dl]) was detected during the study. There was no correlation between fasting glucose and fasting glucagon; 23% had a suppressed fasting glucagon.

Results
Twenty-six candidates were studied – 12 male and 14 female. Twenty candidates were using liraglutide once daily and six candidates, exenatide twice daily. The duration of GLP-1 treatment was between 4–50 months. There was no difference in the characteristics of those who achieved a reduction in HbA1c of ≥1% (n=18) to those who did not (n=8); Table 1.

Cohort correlations
Beta-cell function: Beta-cell function were significantly negatively correlated with HbA1c: HOMA B (r = -0.53, p<0.05); Insulinogenic 30 Index (r = -0.46, p<0.05); Disposition Index 30 (r = -0.53, p<0.05); and 120 minute C-peptide (r = -0.40, p<0.05). (Figures 1a–d.) Fasting glucose was also strongly correlated to HbA1c (r = 0.8, p<0.01). Additionally, HOMA B declined with increasing diabetes duration (r = -0.51, p<0.05).

Insulin sensitivity: There was no correlation between glycemic control, as assessed by HbA1c, and the indices of insulin resistance or sensitivity: HOMA IR (r = 0.373, p=NS) and Matsuda Index (r = -0.07, p=NS). HOMA IR was, nevertheless, correlated with BMI (r = 0.57, p<0.01).

Glucagon: There was no correlation between fasting glucagon and HbA1c (r = 0.15, p=NS).

None of the subjects reported nocturnal hypoglycaemia prior to the LMTT. No episode of hypoglycaemia (blood glucose <4 mmol/L [72 mg/dl]) was detected during the study. There was no correlation between fasting glucose and fasting glucagon; 23% had a suppressed fasting glucagon.

Discussion
Poor glucose control in obese patients with type 2 diabetes treated with GLP-1 agonist is an important clinical problem. Our study suggests that a major factor determining the hyperglycaemia in this patient group is poor beta-cell function rather than hyperglycaemia or insulin resistance.

We assessed beta-cell reserve in the fasting state (HOMA B) and, through an LMTT, first phase insulin response (Ingenix 30), beta-cell function (Mttdi 30) and second phase insulin secretion (120 minutes C-peptide) in patients with various glycemic control on GLP-1 agonists. We used an LMTT to produce a physiological stimulus with a carbohydrate and protein challenge to augment any residual beta-cell reserve and incretin release. LMTTs have been shown to compare well to more ‘gold standard’ tests of beta-cell reserve such as frequently sampled intravenous glucose tolerance tests. LMTT derived indices have been shown to provide reliable estimates for the assessment of insulin secretion and sensitivity in moderate sized clinical trials.

Our results demonstrate that beta-cell reserve is an important metabolic factor for glycemic control in patients on established treatment with GLP-1 agonists. Firstly, HOMA B correlated strongly with HbA1c. HOMA B, a product of fasting glucose and fasting insulin, is partly influenced by the lower fasting glucose in subjects with better glucose control. This is consistent with studies showing that GLP-1 infusions have the ability of lowering blood glucose in the overnight fasting state and not just in the postprandial.
state. First phase insulin release, measured by the Insulinogenic 30 Index, was also strongly negatively correlated with glycemia suggesting that subjects with worse glycemia have poorer first phase insulin release despite therapy with GLP-1 agonist. The LMTT Disposition Index 30, a global measure of insulin effectiveness, was also strongly negatively correlated with glycemia in the cohort. Second phase insulin release, measured by 120 minute C-peptide, also negatively correlated with glycemia in the cohort. This would suggest that those with poorer glycemic control tend to have a worse second phase insulin release.

Hyperglucagonaemia has been a well described feature of type 2 diabetes. In moderately well controlled type 2 diabetes on monotherapy, it has been estimated that glucagon contributes up to 50% of the hyperglycaemia. It should be noted, however, that this estimate has been challenged as too high due to methodological problems. The precise mechanism by which GLP-1 agonists inhibit glucagon release is not fully understood as there do not seem to be GLP-1 receptors on alpha cells.

Fasting glucagon did not correlate with HbA1c in our whole cohort. No episode of hypoglycaemia, which could have stimulated glucagon release, was detected during the study. Our measured fasting glucagon levels are similar to values found in patients with type 2 diabetes on diet or oral hypoglycaemic drugs. Fasting glucagon did not correlate with HbA1c in our whole cohort. No episode of hypoglycaemia, which could have stimulated glucagon release, was detected during the study. Our measured fasting glucagon levels are similar to values found in patients with type 2 diabetes on diet or oral hypoglycaemic drugs. Fasting glucagon did not correlate with HbA1c in our whole cohort. No episode of hypoglycaemia, which could have stimulated glucagon release, was detected during the study. Our measured fasting glucagon levels are similar to values found in patients with type 2 diabetes on diet or oral hypoglycaemic drugs.

Insulin sensitivity has been shown to improve with GLP-1 agonists in some short-term human studies. Insulin resistance would be expected to improve if GLP-1 agonist therapy is associated with loss of fat mass. Our study did not find any significant correlation between HbA1c and insulin sensitivity measures. In conclusion, this study demonstrates that impaired beta-cell function, but neither insulin resistance nor elevated fasting glucagon, are associated with poorer diabetes control in long-term GLP-1 treated patients with type 2 diabetes. Patients with worse beta-cell reserve respond less well to GLP-1 agonists. Although our study design does not allow us to test causality, we infer that if patients with type 2 diabetes treated with oral hypoglycaemic drugs and a GLP-1 agonist have poor glucose control then they require extra measures to enhance insulin action; with our currently available therapies this is likely to entail exogenous insulin. This adds to the argument made for the role of combined GLP-1 agonist with insulin therapy for individuals who fail to reach target glycemic control on GLP-1 agonists only.

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Declaration of interests
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