Application of the intravenous glucose tolerance test and the minimal model to patients with insulinoma: insulin sensitivity (Si) and glucose effectiveness (Sg) before and after surgical excision


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Summary

Background The unmodified frequently sampled intravenous glucose tolerance test (FSIGT) has not previously been used to assess insulin/glucose kinetics in patients with insulinoma.

Objective To measure insulin sensitivity (Si) and glucose effectiveness (Sg) by means of the FSIGT in patients with insulinoma, before and after surgical removal of the tumour.

Subjects and methods FSIGTs were performed in five patients, before and approximately 3 months post-surgery, and in 11 controls. Si and Sg were estimated using Minimal Model computer analysis of dynamic glucose and insulin data.

Results Si was lower in insulinoma patients before, compared with after surgery (3.37 ± 0.62 vs. 6.24 ± 1.09 SE × 10^-4 min^-1 μU^-1 ml, P < 0.05). Sg was similar in patients pre- and post-surgery (3.0 ± 0.67 vs. 2.4 ± 0.6 × 10^-4 min^-1, NS).

Conclusions Insulin sensitivity improves after excision of an insulinoma. Glucose effectiveness is not influenced by chronic hyperinsulinaemia and hypoglycaemia.

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Introduction

Insulinoma is an uncommon condition with little reported of insulin secretion/action and glucose kinetics in these patients. A limited number of studies on small groups have been described, using various indices of insulin sensitivity (Si) as derived from one-step, two-step and three-step euglycaemic hyperinsulinaemic clamps, before and after tumour resection. Glucose-mediated glucose disposal (Sg), which is defined as the ability of glucose at basal insulin to enhance its own utilization and suppress its own endogenous production however, has not been assessed in patients with insulinoma – though it may be considerably affected by circulating plasma insulin levels.

There are several methods of quantifying insulin sensitivity. Only two – the euglycaemic insulin clamp and the frequently sampled intravenous glucose tolerance test (FSIGT) – are widely accepted by consensus as providing an accurate estimate of peripheral insulin resistance.

Two-step hyperinsulinaemic euglycaemic clamps have long been the reference method of measuring Si, and although technically demanding, provide more accurate and precise values for Si than one-step clamps which are easier to perform. Both one-step and two-step clamps however, cannot simultaneously estimate Si and the ability of glucose to self-regulate its level (Sg). To estimate Sg a separate hyperglycaemic basal insulin clamp would be required.

The unmodified FSIGT, on the other hand, is a simpler and shorter means of assessing both Si and Sg with one protocol that has not previously been used in patients with insulinoma. In addition, it enables us to directly evaluate the endogenous insulin response to a stimulatory glucose bolus, without the interference of exogenous insulin. With these considerations in mind, we chose to use the unmodified FSIGT to estimate Si and Sg in patients with insulinoma, before and after surgery.

We know from studies carried out by Bergman, that the product of insulin sensitivity (Si) and insulin secretion is a constant value in healthy individuals. We therefore postulated that if insulin secretion increases, as in patients with insulinoma, then Si should decrease. We also hypothesized that glucose effectiveness (Sg) would be altered in patients with insulinoma, as Sg is known to be influenced by plasma insulin and counter-regulatory hormones.

The aim of our study was to assess Si and Sg in patients with insulinoma, before and after surgical resection, using the unmodified FSIGT.
Table 1. Clinical characteristics of the five insulinoma patients, before and after surgery

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Gender</th>
<th>Pre-operative weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>Time of postoperative FSIGT (months after surgery)</th>
<th>Fasting glucose pre-operative (mmol/l)</th>
<th>Fasting insulin pre-operative (mU/l)</th>
<th>Postoperative Kg* (% min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>47</td>
<td>53</td>
<td>22.5</td>
<td>3</td>
<td>2.9</td>
<td>29</td>
<td>2.43</td>
</tr>
<tr>
<td>Patient 2</td>
<td>72</td>
<td>66</td>
<td>26.8</td>
<td>3</td>
<td>2.1</td>
<td>52</td>
<td>1.18</td>
</tr>
<tr>
<td>Patient 3</td>
<td>39</td>
<td>78</td>
<td>25.8</td>
<td>3.5</td>
<td>3.9</td>
<td>44</td>
<td>2.86</td>
</tr>
<tr>
<td>Patient 4</td>
<td>32</td>
<td>80</td>
<td>32.5</td>
<td>3</td>
<td>2.3</td>
<td>62</td>
<td>0.94</td>
</tr>
<tr>
<td>Patient 5</td>
<td>41</td>
<td>54</td>
<td>20.3</td>
<td>2.5</td>
<td>2.9</td>
<td>8</td>
<td>1.94</td>
</tr>
</tbody>
</table>

*Kg, rate of glucose disappearance.

Table 2. Demographic and anthropometric characteristics of the 11 control subjects

<table>
<thead>
<tr>
<th>Mean age (range) (years)</th>
<th>Mean BMI (range) (kg/m²)</th>
<th>Males/females</th>
</tr>
</thead>
<tbody>
<tr>
<td>42 (40–60)</td>
<td>25.5 (23–28)</td>
<td>8/3</td>
</tr>
</tbody>
</table>

Subjects and methods

Subjects

Five patients, three women and two men, with solitary localized insulinomas who underwent tumour resection at St. Vincent's Hospital Melbourne and 11 controls took part in this study. The main clinical characteristics of those with insulinoma are summarized in Table 1 whilst the demographic and anthropometric data of the control population are summarized in Table 2.

All five patients with insulinoma had no other significant past medical history and were diagnosed using the standard 72-h fast with exclusion of factitious hypoglycaemia. None of the patients had the MEN1 syndrome. None were on any form of medical therapy for insulinoma (such as diazoxide), hormonal contraceptives or hormonal replacement therapy at the time of the FSIGT. The control subjects were healthy volunteers with normal glucose tolerance. They were not on any regular medications and had no first degree relatives with diabetes. The female patients and controls who were pre-menopausal, were in the follicular phase of the menstrual cycle.

The studies were performed with the informed consent of the patients in compliance with the recommendations of the Declaration of Helsinki and the approval of the St. Vincent's Hospital Melbourne Ethics Committee.

Methods

FSIGTs were performed in the five patients with insulinoma before and approximately 3 months after surgery, and results compared with FSIGTs in a group of 11 healthy volunteer controls. Patients were fasted overnight for 10 h, and then given an intravenous bolus of 0.3 g/kg of 50% glucose at 0 min. During the test the patients were asked to lie supine and blood samples were drawn from a warmed upper limb. Blood for glucose and insulin were sampled at -20, -10, -1, 0, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 140, 160 and 180 min. Plasma glucose was measured with an automatic analyser (Yellow Springs Instruments, Greene County, OH) using a glucose oxidase method, CV 3-2% at 4.8 mmol/l and 6-7% at 12.3 mmol/l. Plasma insulin was measured by radioimmunoassay by the method of Albano et al. using charcoal separation of bound from free fractions. The interassay CVs were 7-7% at insulin level 80 mU/l, and 6-8% at 35 mU/l.

Data analysis

Si and Sg were estimated by fitting the Minimal Model of Bergman et al. to the FSIGT glucose and insulin data using the MINMOD MILLENNIUM® computer program. The rate of glucose disappearance (Kg) was calculated as the least-squares slope of the logarithm of the plasma glucose concentrations between 10 and 40 min after the glucose load.

As indices of insulin secretion, we calculated the insulin : glucose ratios at baseline (0 min) and in the interval from 120 to 180 min.

Statistical analysis

Comparisons between the pre- and post-surgery groups were performed using the paired Student’s t-test with the computer software MICROSOFT EXCEL®. Significance was considered at P < 0.05. With coefficients of variation of 15% for estimation of Si and Sg, a difference between the mean values pre- and postoperation of 25% would be detected with a power of 0.8. The insulin : glucose ratio data were not normally distributed, therefore comparisons of preoperative patients with controls were performed using the Kolmogorov–Smirnov two-samples test with the computer software STATA 10.0®.
Results

Postoperatively, all patients achieved complete symptomatic and biochemical resolution, and none developed diabetes, based on fasting glucose and intravenous glucose disposal rate (kg) (Table 1).

Results are quoted as means \pm SE, apart from the insulin:glucose ratios which are quoted as median and range.

Insulin sensitivity (Fig. 1)

The mean Si of the five patients with insulinoma was lower before, compared with after tumour resection (3.37 \pm 0.62 vs. 6.24 \pm 1.09 SE [10^{-4}] min^{-1}uU^{-1}ml^{-1}, P < 0.05).

Si of patients with insulinoma after surgery was similar to the normal controls (4.76 \pm 0.71).

Glucose effectiveness (Fig. 2)

There was no consistent change in Sg in patients pre- and postsurgery (3.0 \pm 0.67 vs. 2.4 \pm 0.60 SE [10^{-4}] min^{-1}, NS) and both were similar to normal controls (2.1 \pm 0.26).

Patterns of insulin response to glucose, before and after surgery (Fig. 3)

Pre-operatively, all patients with insulinoma attained peak insulin levels in response to the intravenous glucose challenge by 3–6 min. In two patients, insulin levels declined in tandem with glucose levels. In the other three patients paradoxical surges of insulin secretion occurred, despite declining levels of glucose. These secretory abnormalities were corrected by surgery in all five patients.

Insulin:glucose ratios (Fig. 4)

The median basal insulin:glucose ratio was higher in preoperative than postoperative patients and normal controls [9.56 (range, 2.81–25.9) vs. 1.42 (1.11–2.84), P < 0.05 and 9.56 (2.81–25.9) vs. 1.54 (0.7–3.3) mU/mmol, P < 0.05, respectively]. However, the ratios in the preoperative group overlapped with those of healthy volunteers.

The median insulin:glucose ratio between 120 and 180 min was also higher in patients with insulinoma before surgery compared with post-resection, and when compared with normal subjects [9.62 (7.6–29.6) vs. 1.75 (0.71–3.52), P < 0.05 and 9.62 (7.6–29.6) vs. 1.82 (1.16–3.92), P < 0.01, respectively]. There was, however, no overlap between values in patients with insulinoma before surgery and control subjects.

Discussion

Our studies show that while insulin sensitivity in patients with insulinoma improves after surgical resection, glucose effectiveness was not influenced by the chronic hyperinsulinaemia and hypoglycaemia found in this disorder.

We chose to evaluate Si and Sg by means of the unmodified FSIGT because of the limitations of euglycaemic clamps. The two-step clamp is mainly carried out in research facilities and while the one-step clamp is not as technically demanding, the intersubject variability of plasma insulin levels at high insulin infusion rates detracts from the accuracy and precision of its insulin sensitivity index. On the other hand, fasting indices such as HOMA and QUICKI while much easier to perform, are of little value in evaluating Si in patients with insulinoma and doubtful accuracy when compared with insulin-modified FSIGTs even in nondiabetic controls. C-peptide was not measured because exogenous insulin was not used in our study and factitious hypoglycaemia had previously been excluded.

Our results demonstrated a significant improvement in insulin sensitivity in all patients within 3–5 months after surgery. Several other groups have examined insulin sensitivity before and after surgical excision, by different methodology, with varying results. While one small one-step hyperinsulinaemic euglycaemic clamp study demonstrated no postoperative change in insulin-mediated glucose disposal (a reflection of Si), an improvement similar to that in our patients has been demonstrated with the use of two-step and three-step clamps.

Our study was unable to demonstrate a significant difference in Si between patients with insulinoma before surgery and normal subjects because of its small sample size; but others have confirmed insulin resistance when compared with controls with the use of clamps. In the largest of these studies, 20 patients with
insulinoma, though insulin-resistant, were found to have the highest Si of a group with syndromes of insulin resistance and hyperinsulinaemia (e.g. obesity, family history of Type 2 Diabetes [T2D], Impaired Glucose Tolerance and T2D).21

While our study did not address the mechanisms of reduced insulin sensitivity in insulinoma, others have hypothesized a compensatory down-regulation of insulin receptors22 (with reduced number and affinity of insulin receptors23,24), or post-receptor defect.42

Alternatively, the counter-regulatory response to hypoglycaemia which can lower Si by as much as 66% during an insulin-modified FSIGT in healthy subjects25 may have a similar effect in insulinoma patients whose basal glucagon and GH levels are also raised during fasting hypoglycaemia.22

The insulinoma tumour provides a unique model allowing us to study the impact of chronic hyperinsulinaemia coupled with chronic hypoglycaemia upon glucose effectiveness. Previous studies indicate43,4,13,14 that Si might be either increased or decreased in

Fig. 3 Comparison of plasma glucose (—O—) and insulin (■—■) profiles during the unmodified FSIGT in insulinoma patients before (a) and after (b) surgery: (a) Individual patient glucose and insulin profiles before surgery; and, (b) Mean glucose and insulin levels for all five patients postoperatively.
Patients with insulinoma have considerable heterogeneity with regards to absolute insulin levels, secretory patterns\textsuperscript{19} and C-peptide response during suppression\textsuperscript{20,21} stimulation tests.\textsuperscript{22} Nauck et al.\textsuperscript{22} has delineated three patterns of insulin secretion in insulinoma patients during hyperinsulinaemic clamps and while there have been case reports of tumours responding to oral glucose stimuli with supra-physiological surges of insulin,\textsuperscript{23} little is known about the in vivo response of neoplastic β cells to intravenous glucose in humans. Using the unmodified FSIGT to analyse secretory patterns (Fig. 3a), we found that preoperatively, all our patients had a surge of first phase insulin secretion within 6 min of the glucose bolus, consistent with glucose-responsiveness of the tumour or the residual normal β cells in the pancreas. Later in the course of the preoperative FSIGT, we observed one of two distinct profiles: (i) a decline of inappropriately high insulin levels in tandem with glucose, or (ii) peaks of insulin secretion despite falling glucose values.

Having observed this mimicry of physiological first phase secretion in insulinoma patients, we conjectured that the FSIGT might serve as a provocative test in those unable to tolerate prolonged fasts. The traditional, labour- and cost-intensive 72-h fast is considered necessary for diagnosis because synchronized insulin hypersecretion occurs randomly or in response to undefined stimuli, such as exercise. An intravenous glucose bolus however, would standardize conditions and temporarily synchronize insulin release in these patients: the ensuing supra-physiological insulin peak coupled with a fast of 13 h (10 h overnight +3 h of the FSIGT) would result in higher insulin levels and concomitantly lower glucose levels during the last hour of the procedure. In our study, the mean insulin: glucose ratio between 120 and 180 min showed a clear separation between normal and pathological, with no overlap. Thus, the discriminatory power of this variable was better than that observed with the basal insulin: glucose ratio. Insulin: glucose ratios have been used with varying success in the past to differentiate patients with insulinoma from healthy subjects.\textsuperscript{17-20} The small number of subjects in our study however, precludes any conclusion on its utility as a diagnostic parameter. Further exploratory studies in larger samples of patients with insulinoma, as well as other groups with hyperinsulinaemia coupled with insulin resistance (e.g. obesity, T2D), are required to establish the sensitivity and specificity of this variable.

In conclusion, our frequently sampled intravenous glucose tolerance test studies in patients with insulinoma showed that insulin sensitivity improves significantly after surgical resection of the tumour. In contrast, glucose effectiveness in patients with insulinoma is not affected by their chronic hyperinsulinaemia and hyperglycaemia.

References


