Improvement in insulin sensitivity following intensive insulin therapy and association of glucagon with long-term diabetes remission

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Abstract
Objective: To investigate the role of the acute glucagon response in the long-term remission of newly diagnosed type 2 diabetes mellitus following short-term intensive insulin therapy (IIT).
Methods: Ten patients with newly diagnosed type 2 diabetes mellitus received IIT. Intravenous glucose tolerance tests and the clamp technique were performed pre- and post-IIT. Remission was defined as maintenance of target glycaemic control without anti-diabetic agents for 1 year.
Results: The remission rate was 50% (5/10). There were no differences in the acute insulin response or glucose infusion rate between groups. The acute glucagon response (AGR) in the remission group pre-IIT was significantly higher than that in the non-remission group (mean 163.02 pg/mL/min vs. mean 16.29 pg/mL/min). The mean AGR post-IIT was lower in the remission group than that in the non-remission group (0 pg/mL/min vs. 19.91 pg/mL/min). Spearman analysis indicated that the AGR pre-IIT and the change in the AGR were correlated with remission (r = 0.731).
Conclusion: The insulin-mediated glucose disposal rate was significantly improved with the normalization of blood glucose levels following transient IIT. Subjects with a higher AGR pre-IIT and a greater AGR decrease post-IIT displayed a greater likelihood of long-term remission.

Keywords
type 2 diabetes mellitus, glucagon, intensive insulin therapy, long-term remission, insulin sensitivity

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Introduction
Short-term intensive insulin therapy (IIT) can induce long-term remission of newly diagnosed type 2 diabetes mellitus (T2DM), which
sheds light on a new approach to treatment of patients with T2DM. However, the mechanism underlying long-term remission remains unclear. Previous studies have demonstrated that T2DM duration is related to long-term remission. Other studies have reported that insulin resistance and first-phase insulin secretion both improved following IIT, but neither were related to long-term remission. Furthermore, only a few studies have examined the effect of IIT on changes in insulin sensitivity using a hyperinsulinaemic-euglycaemic clamp in patients with newly diagnosed T2DM. One aim of the present study was to investigate the impact of short-term continuous subcutaneous insulin infusion (CSII) therapy on insulin sensitivity and insulin secretion assessed by clamp technique and intravenous glucose tolerance test (IVGTT), respectively.

As the pathophysiology of diabetes includes dual hormone abnormalities, we proposed that changes in pancreatic alpha-cell function may correlate with long-term T2DM remission. The present study investigated the relationship between the acute glucagon response (AGR) and long-term T2DM remission following short-term IIT. Furthermore, we have discussed the role of the pancreatic alpha cell in long-term remission of newly diagnosed T2DM.

**Patients and methods**

**Subjects**

As the hyperinsulinaemic-euglycaemic clamp is cumbersome and labour-intensive, 10 patients with newly diagnosed T2DM were recruited to the study. The study protocol was approved by the institutional review board of China-Japan Friendship Hospital. Written informed consent was obtained from each participant at enrolment.

Inclusion criteria of the subjects included: newly diagnosed T2DM (according to WHO diagnostic code, 1999); duration of disease shorter than 12 months; negative insulin autoantibody, glutamate decarboxylase antibody and insulin cellular antibody; naïve to anti-diabetic medication; fasting plasma glucose (FPG) levels >11.1 mmol/L; aged between 25 and 70 years; and acute diabetic complications and severe cardiac, hepatic and renal disease absent.

**Measurements**

Fasting blood samples were drawn from the 10 hospitalized subjects to analyse blood glucose levels (using the glucose oxidase method). An IVGTT was performed followed by a hyperinsulinaemic-euglycaemic clamp the next day. The 2-week IIT with CSII was then initiated with the prerequisite that blood glucose levels should be restored to normal (FPG <6.1 mmol/L and 2 h postprandial glucose for all three meals <8.0 mmol/L) within 3 days. IVGTTs and hyperinsulinaemic-euglycaemic clamps were performed 24 h after IIT termination.

**IVGTT procedures.** Following a 10-h fast, venous detaining needles were placed into the ulnar veins in both arms of the patient. One needle was used for rapid injection of 50% glucose solution (25 g) and the other for drawing blood. At -15, 0, 2, 3, 5 and 10 min, blood samples were obtained for the determination of glucose, insulin and glucagon concentrations. Specific insulin levels were measured by radioimmunoassay (DSL Co, USA).

The first-phase insulin secretion rate, also known as the acute insulin response (AIR), was calculated by determining the sum of the increments under the curve during the first 10 min of the IVGTT using the trapezoidal algorithm. The AIR was also directly calculated by determining the difference between the insulin peak value during the first 10 minutes of the IVGTT and the fasting insulin value (mU/L).
Glucagon levels were measured by radio-immunoassay (Linco Research, USA), with an intra-assay coefficient of 4.6% and an inter-assay coefficient of 7.3%. The AGR was determined by calculating the size increment under the glucagon concentration curve, which could be achieved by determining the under-curve size increment during the first 10 minutes of the IVGTT with a trapezoidal algorithm.6

Hyperinsulinaemic-euglycaemic clamp procedures. In this test, the German EKF clamp system was employed. The test started at 7:30 a.m. by infusing human insulin (40 U/ml humulin R; Lily Co, USA) NS solution and 20% glucose solution through the detaining needle into one ulnar vein. The dorsum manus vein on the other side with a detaining needle inserted was prepared for extracting arterialized venous blood after heating the arm with an infrared heater to 50–55°C. Human insulin solution was infused at a rate of 3 mU/kg/min during the first 5 min and 2 mU/kg/min during the next 5 min to rapidly elevate blood insulin levels. For the remaining 140 min, the infusion rate was maintained at 1 mU/kg/min. Blood samples were obtained every 5 min from the arterialized vein to measure plasma glucose concentrations (Biosen 5030 blood glucose detector; EKF Co. Germany). The 20% glucose infusion rate was adjusted periodically to maintain blood glucose levels (approximately 5 mmol/L). Blood samples were obtained every 20 min for insulin radio-immunoassay analysis (DSL Co). Because FPG levels in patients with T2DM were elevated, particularly before IIT, an infusion rate of 3 mU/kg/min insulin was adopted to lower blood glucose levels to approximately 5.5–6 mmol/L 1–2 h before initiating the 150-min clamp test.5

The glucose infusion rate (GIR) was calculated from the mean GIR value during the final 1 h of the steady state.

Follow-up
The patients were maintained on lifestyle modifications, including diet, exercise and self-monitoring of blood glucose levels. All patients were followed for at least 1 year. Long-term remission was defined by two key points. First, patients did not receive any anti-diabetic agents. Second, patients maintained good control of blood glucose levels (FPG <7 mmol/L and 2-h postprandial glucose <10 mmol/L) after 1 year by self-monitoring. Furthermore, patients documented the same level after 1 year as that observed following 2-week IIT.7

Statistical analyses
Normally distributed data were presented as mean ± SD. Paired t tests were used for comparison between parameters before and after IIT. One-way ANOVAs were used for comparisons between the remission and non-remission groups. A Spearman’s rank correlation test was used when appropriate. All statistical analyses were performed with SPSS 16.0 software.

Results
Insulin secretion function
During the 2-week IIT, nine of 10 subjects displayed normalized blood glucose levels after 3 days, and the remaining subject after 5 days. No patients had any adverse reactions. Subject characteristics at baseline are displayed in Table 1. The insulin secretion curve indicated that first-phase insulin secretion was not present in all patients with T2DM before IIT. After 2-week IIT, blood glucose levels were all nearly normalized, and insulin secretion was restored in all patients, though to varying degrees. Patients nos. 2, 3, 9 and 10 displayed the most dramatic improvements (Table 2). Compared with the AIR
before IIT, the AIR increased significantly following IIT (0.83 ± 1.96 mU/L vs. 7.63 ± 4.73 mU/L, \( P < 0.005 \)).

**Determination of insulin sensitivity**

Hyperinsulinaemic-euglycaemic clamp parameters in the steady state were analysed. Plasma glucose concentrations before and after acute IIT were 5.03 ± 0.06 mmol/L and 5.01 ± 0.05 mmol/L, respectively. The plasma glucose variation coefficients were 4.46% and 5.79%, respectively. Insulin concentrations were 83.50 ± 11.39 mU/L and 81.80 ± 23.31 mU/L, respectively.

The mean GIR in the 10 patients with newly diagnosed T2DM before IIT was 2.30 ± 0.81 mg/kg/min. Following IIT, the GIR increased significantly to 5.33 ± 1.43 mg/kg/min (\( P < 0.0001 \), compared with that before IIT). Patients nos. 2, 4, 6 and 7 displayed the highest GIR restoration,
which was more than 70% of the normal value (Table 2).

**Follow-up analysis**

**Comparison of demographic data between the remission and non-remission groups.** At 1-year follow-up, five of 10 patients had achieved long-term remission (remission rate: 50.0%). In the non-remission group, three patients received one oral anti-diabetic agent, one patient received two oral agents, and one patient received insulin treatment. Age, sex, body mass index (BMI), waist circumstance and T2DM duration in the remission group were not significantly different from those in the non-remission group. FPG levels decreased significantly post-IIT compared with those pre-IIT in both the remission and non-remission groups ($P < 0.05$). However, FPG levels did not differ between the remission and non-remission groups either pre-IIT or post-IIT (Table 1).

**Comparison of plasma glucagon levels during the IVGTT between the remission and non-remission groups.** Before IIT in the remission group, at 2, 3, 5 and 10 min post-glucose load, plasma glucagon levels were higher than fasting plasma glucagon levels. However, following IIT, post-glucose load glucagon levels decreased significantly relative to fasting plasma glucagon levels. In the non-remission group, plasma glucagon levels were not significantly altered following IIT (Figure 1).

**Comparison of variables between the remission and non-remission groups before and after treatment.** The AIR, which reflects first-phase insulin secretion, and GIR, which reflects insulin sensitivity, were not significantly different between the two groups before IIT. However, before IIT, the AGR differed significantly between the remission and non-remission groups (163.02 pg/mL/min and 16.29 pg/mL/min, respectively; $P < 0.05$). Following IIT, the GIR and AIR increased significantly in both groups ($P < 0.01$), and the AGR decreased significantly in the remission group ($P < 0.01$).

![Figure 1](image_url)
Table 3. Comparison of clinical variables between the remission and non-remission groups before and after intensive insulin therapy (mean ± SD).

| Group             | n | Time  | GIR (mg/kg/min) | AIR (mU/L/min) | AGR (pg/mL/min) |
|-------------------|---|-------|----------------|----------------|-----------------|
| **Remission group** | 5 | pre-IIT | 2.02 ± 0.83     | 1.22 ± 1.76    | 5.10 ± 0.60*    |
|                   | 5 | post-IIT | 5.39 ± 1.76*   | 4.01 ± 1.33*   | 0.00 ± 0.00*    |
| **Non-remission group** | 5 | pre-IIT | 2.58 ± 0.77     | 1.81 ± 1.24    | 2.85 ± 1.86     |
|                   | 5 | post-IIT | 5.26 ± 1.22*   | 3.62 ± 1.05*   | 3.04 ± 2.00     |

GIR, glucose infusion rate; AIR, acute insulin response; AGR, acute glucagon response; IIT, intensive insulin therapy.

*The number in parentheses is the retro natural logarithm of means of this parameter vs. the non-remission group during the same period.

#P < 0.05; vs. pre-treatment in the same group, *P < 0.01

Table 3. The GIR and AIR were not significantly different between the two groups after IIT. However, the decrease in the AGR in the remission group was significantly higher than that in the non-remission group (185.06 pg/mL/min and −26.06 pg/mL/min, respectively; P < 0.05).

Spearman correlation analysis revealed that T2DM duration, BMI, AIR, GIR and FPG levels were not associated with long-term remission. In contrast, the pre-IIT AGR and AGR reduction post-IIT were associated with long-term remission (r = 0.731, P = 0.016).

**Discussion**

Beta cell dysfunction and insulin resistance underlie the primary pathophysiological mechanisms involved in T2DM development. In some patients with newly diagnosed T2DM, a “honeymoon period”, which is characterized by long-lasting normal or near normal glycaemic control without anti-diabetic medications, can be induced with short-term multiple insulin injection therapy or insulin pump therapy. It has been reported that in patients who gained long-term T2DM remission, first-phase insulin secretion and insulin secretion function based on the homeostatic model assessment of beta cell function calculation were significantly improved. However, there are some limitations to surrogate indices based on the homeostatic model assessment of insulin resistance or oral glucose tolerance tests when they are applied to assess insulin sensitivity. To our knowledge, the present study is the first to evaluate insulin sensitivity using the hyperinsulinaemic-euglycaemic clamp technique (the gold standard for the evaluation of insulin sensitivity in vivo) in IIT studies.

In the present study, when blood glucose levels were normalized, both insulin secretion and insulin sensitivity improved in eight of 10 patients following IIT. On average, the AIR increased eight-fold above baseline, while the GIR increased two-fold above baseline. The results indicate that ‘glucose toxicity’ is one of the primary pathophysiological mechanisms underlying the deterioration of insulin sensitivity and pancreatic beta-cell function in newly diagnosed T2DM. Early IIT use to stabilize blood glucose levels and induce long-term remission may be an important strategy for treating newly diagnosed T2DM.

Surprisingly, the AIR and GIR were not associated with long-term T2DM remission.
This finding was consistent with previous reports\(^2\)\(^3\) and was more convincingly evaluated in the present study, as insulin sensitivity was assessed using the clamp technique. Other factors may have a stronger influence on long-term remission. Therefore, we assessed additional parameters.

Unlike previous studies, we investigated changes in glucagon levels before and after IIT. The results indicated that the AGR before IIT and the reduction in AGR following IIT were positively correlated with long-term remission, a finding that had not been previously reported. The AGR, which reflects alpha-cell function, may be one of the determinants for long-term remission. Evidence from animal and human studies has indicated that increased glucagon secretion plays an important role in the initiation and maintenance of hyperglycaemia in patients with T2DM. Glucagon promotes fasting and postprandial hyperglycaemia through glycogenolysis, gluconeogenesis, ketogenesis and lipolysis.\(^10\)

Glucagon is a glucose counterregulatory hormone that is secreted during hypoglycaemia and is suppressed when plasma glucose levels range from 4 mmol/L to 6 mmol/L in healthy individuals.\(^11\) However, in patients with T2DM, glucagon secretion is not suppressed during hyperglycaemia and is instead enhanced. This phenomenon is due to the loss of insulin-producing beta cells surrounding the pancreatic alpha cells. Insulin is a key regulatory factor for controlling glucagon secretion by alpha cells.\(^12\) Because beta cells in patients with T2DM have defects in glucose sensing,\(^13\) glucose-stimulated insulin secretion is impaired during hyperglycaemia. The loss of insulin secretion reduces the suppressive effect of insulin on glucagon secretion, thereby increasing glucagon release. In addition, alpha cells in patients with T2DM are insulin resistant,\(^14\) which further reduces the inhibitory effect of insulin on the AGR. A recent study demonstrated that acute IIT can reduce post-challenge hyperglucagonaemia but does not normalize the alpha-cell response. This effect does not appear to be mediated by the improvement in beta-cell function that is induced by IIT.\(^15\)

In the present study, the AGR was elevated in the remission group before IIT and decreased significantly after 2-week IIT. In contrast, the AGR was resistant to glucose stimulation and did not change significantly during IIT in the non-remission group. It is unclear whether alpha-cell dysfunction in T2DM is solely caused by impaired beta-cell function.\(^10\),\(^15\) The mechanism by which changes in alpha-cell function correlate with remission is also unclear.

In conclusion, the AIR and GIR improved significantly after 2-week IIT in patients with newly diagnosed T2DM. Individuals with a higher AGR were more likely to attain long-term remission. This finding is important for choosing appropriate subjects to receive short-term IIT to improve treatment outcomes in patients with newly diagnosed T2DM. One advantage of the present study is that we used complicated and reliable techniques to assess insulin sensitivity and beta- and alpha-cell function. However, these methods are complex, time-consuming and expensive and thus confined the sample size of the current study. Accordingly, this study had a modest sample size, no control group and no covariate adjustment. Therefore, results should be confirmed by randomized controlled trials with a larger sample size.

**Declaration of conflicting interest**

The authors declare that there is no conflict of interest.

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