Usefulness of the insulin tolerance test in patients with type 2 diabetes receiving insulin therapy

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ABSTRACT

Aims/Introduction: To establish the validity of the plasma glucose disappearance rate (KITT), derived from an insulin-tolerance test (ITT), for evaluating the insulin sensitivity of patients with type 2 diabetes after insulin therapy.

Materials and Methods: In the first arm of the study, 19 patients with poorly controlled diabetes were treated with insulin and underwent an ITT and a euglycemic clamp test (clamp-IR). The relationship between the insulin resistance index, as assessed by both the clamp-IR and KITT tests, was examined. In the second arm of the study, the relationships between KITT values and various clinical parameters were investigated in 135 patients with poorly controlled diabetes, after achieving glycemic control with insulin.

Results: In study 1, a close correlation between KITT and the average glucose infusion rate during the last 30 min of the standard clamp-IR test (M-value) was noted (P<0.001). In study 2, body mass index (P=0.0011), waist circumference (P=0.0004), visceral fat area (P=0.0011) and the log-transformed homeostasis model assessment of insulin resistance value (P=0.0003) were negatively correlated with the log-transformed KITT. High-density lipoprotein cholesterol (P=0.0183), low-density lipoprotein cholesterol (P=0.0121) and adiponectin (P=0.0384) levels were positively correlated with the log-transformed KITT.

Conclusions: The ITT is a valid and useful test for evaluating the insulin sensitivity of patients with diabetes, even after treatment with insulin.

INTRODUCTION

Type 2 diabetes mellitus is caused by impaired insulin secretion and increased insulin resistance. The evaluation of insulin resistance and β-cell function is essential for an understanding of the disease condition, and for administering appropriate pharmacological treatment. Furthermore, insulin resistance is a valuable parameter for measurement because of its potential as a marker of increased cardiovascular risk. The ‘gold standard’ test for evaluating insulin resistance is the euglycemic clamp (clamp-IR) test, but this test is costly and lengthy to carry out. Therefore, the use of the clamp-IR test is generally limited to research projects, and is difficult to carry out at most medical institutions. Fortunately, a variety of other methods is available for evaluating insulin sensitivity. For regular, clinical use, a simple and safe method is desirable for the evaluation of insulin sensitivity.

The insulin tolerance test (ITT) is a simple and convenient in vivo method for evaluating insulin action. The plasma glucose disappearance rate (KITT), derived from the ITT, correlates well with clamp-IR test results in subjects with normal glucose tolerance and well-controlled type 2 diabetes. However, the reproducibility of results might be lower in individuals with poorly controlled fasting plasma glucose (FPG); KITT has previously shown an inverse correlation with FPG concentration. The lack of correlation is associated with the known insulin-secreation defects and worsened insulin resistance associated with hyperglycemia. This phenomenon, called glucotoxicity, is partly reversible, and is one of the reasons why glycemic control is required before the evaluation of insulin sensitivity in diabetic individuals.
The ITT is a simple method for the evaluation of insulin sensitivity, but it is also difficult to apply to patients undergoing insulin treatment, because the subcutaneously injected insulin might affect serum insulin and glucose homeostasis. Although insulin resistance evaluations are necessary in insulin users, insulin resistance can only be evaluated in these patients after the minimization of the effects of the subcutaneously injected insulin.

The aim of the present study was to validate KITT values in patients with insulin-induced glycemic control. First we evaluated the correlation of KITT and M-values in patients on insulin therapy (study 1). Then the validity of KITT for representing insulin resistance was investigated in patients with various clinical and biological parameters associated with diabetes to determine the clinical utility of the KITT value (study 2).

MATERIALS AND METHODS

Study 1

Between 2001 and 2006, 19 Japanese type 2 diabetic patients (12 men and 7 women; age 53.6 ± 14.9 years; body mass index [BMI] 23.3 ± 5.5 kg/m²; hemoglobin A1c [HbA1c] concentration 8.7 ± 1.2%) were admitted to Osaka University Hospital, Osaka, Japan, for glycemic control. The clinical characteristics of the patients are summarized in Table 1.

On admission, all oral hypoglycaemic agents were withdrawn, and all patients were started on a diet (25–30 kcal/kg standard bodyweight-day) and insulin (regular or ultrarapid insulin before each meal) for at least 2 weeks until their FPG levels reduced below 126 mg/dL. Neutral protamine Hagedorn insulin before each meal) for at least 2 weeks until their FPG <126 mg/dL after treatment, even though their plasma glucose, before sleeping, was <126 mg/dL. When the FPG decreased to <126 mg/dL after treatment, insulin sensitivity was evaluated by KITT determination and a clamp-IR test (M-values); the correlation between the KITT and M-values were subsequently investigated. Then we investigated the glucose curves divided into two groups (higher and lower degree of insulin resistance evaluated by euglycemic–hyperinsulinemic clamp) with an insulin tolerance test. The glucose levels were expressed as ratios of the value to 0 min.

The ITT was carried out before breakfast, after an overnight fast. Medication of patients on NPH insulin was switched to sulfonlurea (glibenclamide 1.25 or 2.5 mg) before going to sleep on the night before the test. Venous blood samples were collected for measurement of plasma glucose before, and at 3, 6, 9, 12 and 15 min after an intravenous bolus injection of regular insulin (Novorin R, 0.1 U/kg bodyweight; Novo Nordisk, Bagsvaerd, Denmark). 15 minutes after insulin injection, the test was terminated by glucose injection. The KITT was calculated from the linear slope of the plasma glucose concentration curve, between 3 and 15 min, as described previously.

The euglycemic–hyperinsulinemic clamp test was carried out according to the method of DeFronzo et al., with a slight modification with the use of an artificial pancreas (model STG-22; Nikkiso, Tokyo, Japan). Briefly, the test consisted of a 120-min euglycemic–hyperinsulinemic clamp period, during which the patients received a constant infusion of regular insulin (1.45 mU/[kg·min]; Eli Lilly, Indianapolis, IN, USA), and the test was terminated by glucose injection. The KITT determination was carried out 6, 9, 12 and 15 min after an intravenous bolus injection of regular insulin (Novorin R, 0.1 U/kg bodyweight; Novo Nordisk, Bagsvaerd, Denmark). 15 minutes after insulin injection, the test was terminated by glucose injection. The KITT was calculated from the linear slope of the plasma glucose concentration curve, between 3 and 15 min, as described previously.

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Table 1 | Characteristics of the participants in study 1

| Total | Lower degree of insulin resistance | Higher degree of insulin resistance |
|-------|-----------------------------------|-----------------------------------|
| n (Males/females) | 19 (12/7) | 9 (5/4) | 10 (7/3) |
| Age (years) | 53.6 ± 14.9 | 60.6 ± 12.1 | 47.4 ± 14.9 |
| Bodyweight (kg) | 60.0 ± 19.1 | 60.7 ± 14.3 | 59.3 ± 23.4 |
| BMI (kg/m²) | 23.3 ± 5.5 | 230.0 ± 33.3 | 236.2 ± 7.2 |
| HbA1c (%) | 8.7 ± 1.22 | 84.8 ± 0.90 | 89.5 ± 1.47 |
| FPG (mg/dL) | 120.0 ± 15.1 | 115.5 ± 17.1 | 123.4 ± 13.1 |
| F-CPR (ng/mL) | 1.77 ± 0.81 | 1.66 ± 0.44 | 1.87 ± 0.16 |
| Insulin dose (U/day) | 272 ± 279 | 160 ± 58 | 373 ± 36.0 |

Data are expressed as means ± standard deviation. Data were collected after glycemic control, except for that on hemoglobin A1c (HbA1c) levels, BMI, body mass index; eVFA, estimated visceral fat area; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; F-IRI, fasting immunoreactive insulin; U-CPR, urain C-peptide immunoreactivity; ΔCPR, difference in C-peptide levels from the glucose stimulation test. *Significant differences were observed between male and female (P < 0.05).
an exogenous glucose infusion to maintain blood glucose levels at 100 mg/dL and a desired steady-state serum insulin level of 100 μU/mL. When the rate of exogenous glucose infusion reached a steady-state level, we evaluated insulin sensitivity as the average glucose infusion rate over a 30-min period (M-value).

Study 2
Between 2001 and 2008, the 135 Japanese patients with poorly controlled type 2 diabetes (65 men and 70 women), who were admitted to Osaka University Hospital for glycemic control, were enrolled in the present study. We investigated the relationship between KITT values and various clinical parameters in patients with poorly controlled diabetes after glycemic control with insulin. The clinical characteristics of the patients are listed in Table 2. The height and waist circumference of each individual was measured in a standing position, and the visceral fat area was estimated by bioelectrical impedance analysis (BIA), as previously described. On admission, 50.3% of participants were treated with antihypertensive agents, and 42.2% were treated with hypolipidemic agents. These agents were continued until glycemic control improved. Additionally, on admission, the patients were being treated by diet alone (n = 18, 13.3%), diet and hypoglycemic agents (n = 96, 71.1%) or diet and insulin (n = 21, 15.5%). After admission, any oral hypoglycemic agents were withdrawn, and all patients were treated with diet (25–30 kcal/kg standard body-weight-day) and insulin. Only regular or ultrarapid insulin was used before each meal for at least 2 weeks, until the FPG level decreased to <126 mg/dL. When the FPG was >126 mg/dL, and the plasma glucose level was <126 mg/dL before going to bed, NPH insulin was added to the therapeutic regimen, before the patient went.

The ITT was carried out before breakfast, after an overnight fast, as described for study 1. The investigation examined the relationship between the log-transformed KITT values and various clinical parameters, including age, BMI, waist circumference, estimated visceral fat area (eVFA), systolic blood pressure; diastolic blood pressure, log-transformed triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, HbA1c, urinary C-peptide immunoreactivity (CPR), adiponectin, changes in CPR (ΔCPR) from the glucagon-stimulation test and log-transformed homeostasis model assessment of insulin resistance (HOMA-IR) scores.

The HOMA-IR was calculated using the following formula: HOMA-IR = FPG (mg/dL) × fasting immunoreactive insulin (μU/mL) / 405. Before HOMA-IR was calculated, medication was switched to sulfonylurea (glibenclamide 1.25 or 2.5 mg), instead of NPH insulin, the night before the measurement to minimize the influence of long-acting insulin.

A glucagon-stimulation test was carried out with an intravenous infusion of 1 mg glucagon (Novo Nordisk Pharma, Tokyo, Japan) after an overnight fast. Blood samples were collected at 0 and 6 min for measurement of CPR; ΔCPR were also calculated as the difference between the two values. Daily urine samples were collected for the measurement of urinary CPR. Venous blood samples were collected before breakfast to measure low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride and adiponectin levels. Plasma adiponectin levels were determined with an adiponectin enzyme-linked immunosorbent assay (Otsuka Pharmaceuticals, Tokushima, Japan), as described previously. HbA1c values are expressed in National Glycohemoglobin Standardization Program values using the equation table between Japan Diabetes Society and National Glycohemoglobin Standardization Program values.

Patients who were found to possess anti-insulin antibodies, which might influence glucose homeostasis, were excluded from the studies. Written informed consent was obtained from all participants, and the study was approved by the ethics committee of Osaka University.

Statistical Analysis
Data are expressed as means ± standard deviation (SD). The statistical difference between two groups in insulin tolerance test was determined by two-sided Student’s t-test. Pearson’s correlation coefficient analysis was used to assess the relationship between HOMA-IR and the different variables. A P-value <0.05 was considered significant. All analyses were carried out using Statview 5.5 (SAS Institute, Cary, NC, USA).

RESULTS
Study 1
The mean insulin dose used to induce glycemic control was 27.2 ± 27.9 U/day, and the FPG improved from 181.1 ± 45.0 to 120.0 ± 15.1 mg/dL. A total of 10 participants required NPH insulin for glycemic control and received sulfonylurea, instead of NPH, the night before the ITT. After treatment with insulin, the KITT was 1.88 ± 1.13%/min (range 0.32–5.05%/min). The average glucose infusion rate during the last 30 min of the standard clamp-IR test resulted in an M-value of 4.97 ± 1.96 (range 1.52–8.55 mg/kg/min). The correlation between the KITTs and M-values was significant (r = 0.790, P < 0.001; Figure 1).

During the insulin tolerance test, plasma glucose declined from 100% (0 min) to 99.4 ± 1.8% (3 min), 96.4 ± 2.8% (6 min), 92.3 ± 5.1% (9 min), 86.8 ± 7.1% (12 min) and 81.3 ± 9.3% (15 min; Figure 2a). The higher degree of insulin resistance group during euglycemic–hyperinsulinemic clamp consisted of 10 patients, and lower degree of insulin resistance group consisted of nine patients. The plasma glucose of the higher degree of insulin resistance group declined from 100% (0 min) to 99.4 ± 2.1% (3 min), 96.9 ± 2.7% (6 min), 93.9 ± 4.3% (9 min), 89.6 ± 5.5% (12 min) and 84.7 ± 6.9% (1 min). The plasma glucose of the lower degree of insulin resistance group declined from 100% (0 min) to 99.3 ± 1.6% (3 min), 95.8 ± 3.1% (6 min), 90.5 ± 5.6% (9 min), 83.6 ± 7.7% (12 min) and 77.7 ± 10.6% (15 min). In glucose
curves, no difference between the two groups was detected (Figure 2b). In contrast, the KITT value of the higher degree of insulin resistance group was significantly less than that of the lower degree of insulin resistance group (1.37 ± 0.60%/min vs 2.44 ± 1.33%/min, \( P = 0.0334 \)).

Study 2

After treatment, the mean FPG of the 123 patients improved from 178.3 ± 46.8 to 114.9 ± 18.4 mg/dL. The insulin dose used for glycemic control was 22.0 ± 11.7 U/day; NPH insulin was used in 59 patients for glycemic control, with sulfonylurea being substituted for NPH insulin during the night before the ITT. The post-treatment KITT was 1.77 ± 1.14%/min (range 0.39–6.16%/min). The body mass index \( (r = -0.279, P = 0.0011) \), waist circumference \( (r = -0.318, P = 0.0004) \), visceral fat area \( (r = -0.345, P = 0.0011) \) and log-transformed HOMA-IR \( (r = -0.307, P = 0.0003) \) were all negatively correlated with log-transformed KITT; levels of HDL-C \( (r = 0.204, P = 0.0183) \), LDL-C \( (r = 0.216, P = 0.0121) \) and adiponectin \( (r = 0.206, P = 0.0384) \) were positively correlated with log-transformed KITT (Table 3; Figure 3). A significant correlation was not observed between KITT and HbA1c, blood pressure, triglyceride level or insulin secretion capacity.

In the analysis of sex, there was no difference between men and women except for HDL-C, urinary CPR and serum adiponectin (Table 2). In women, the body mass index \( (r = -0.396, P = 0.0006) \), waist circumference \( (r = -0.404, P = 0.0012) \), visceral fat area \( (r = -0.358, P = 0.0135) \) and log-transformed HOMA-IR \( (r = -0.382, P = 0.001) \) were all negatively correlated with log-transformed KITT; levels of HDL-C \( (r = 0.247, P = 0.0377) \), LDL-C \( (r = 0.264, P = 0.0264) \) and adiponectin \( (r = 0.326, P = 0.0151) \) were positively correlated with log-transformed KITT. In men, visceral fat area \( (r = -0.368, P = 0.0213) \) and log-transformed HOMA-IR \( (r = -0.267, P < 0.001) \),
P = 0.0329) were all negatively correlated with log-transformed KITT (Table 3).

**DISCUSSION**

Insulin resistance is a key component of type 2 diabetes, and is also associated with obesity, especially visceral fat obesity\(^2\), hypertension\(^2\), dyslipidemia\(^2\), and hypoadiponectinemia\(^2\). Furthermore, the abnormalities associated with insulin resistance have been suggested to increase the risk of cardiovascular disease\(^4\). Therefore, the evaluation of insulin resistance in type 2 diabetes patients is necessary to provide the most suitable treatment to reduce insulin resistance, and control the risk of cardiovascular disease. KITT is the simplest in vivo test of dynamic insulin action that is widely available, and the present study confirmed the validity of KITT for the evaluation of insulin sensitivity in patients with poorly controlled type 2 diabetes, after insulin therapy.

Study 1 showed a significant correlation between KITT and M-values, even in patients with poorly controlled diabetes, after they were treated with insulin. KITT also correlated well with the M-value in patients with both high and low insulin resistance. Furthermore, this relationship was not dependent on a patient’s need for long-acting insulin (NPH) to maintain glycemic control. Therefore, these results suggest that KITT appropriately reflects insulin sensitivity in type 2 diabetic patients, under optimized glycemic control with insulin.

Insulin dose tended to be more and age tended to be younger in the group of higher degree of insulin resistance during euglycemic–hyperinsulinemic clamp than in the group of lower-degree of insulin resistance, but a significant difference was not recognized. In glucose curves during ITT, no difference between the groups of higher and lower degree of insulin resistance was detected. However, the KITT value of the higher degree of insulin resistance group was significantly less than that of the lower degree of insulin resistance group. These results further indicate the usefulness of KITT.

In study 2, the relationships between the log-transformed KITT and various clinical parameters were defined. These parameters, except HbA1c, were evaluated after glycemic control was achieved, because the patient’s ‘basal’ state was presumed to be approximated after the correction of any glucotoxicity. In the present study, the log-transformed KITT value correlated with various clinical parameters associated with obesity, including BMI, waist circumference and eVFA, which are parameters of body composition, as well as HDL-C and adiponectin levels, which are parameters associated with obesity\(^2\). These results suggest that insulin resistance, assessed by KITT, is also associated with obesity in patients with poorly controlled type 2 diabetes after insulin therapy.

Although 42.2% of the patients were being treated with hypolipidemic agents on study entry, the log-transformed KITT values were still observed to be correlated with HDL-C levels. These results emphasize the validity of the KITT values for reflecting insulin resistance, even in patients with poorly controlled type 2 diabetes, after insulin therapy.

The log-transformed KITT values correlated well with the log-transformed HOMA-IR values, another method for evaluating insulin sensitivity\(^2\). HOMA-IR has also been shown to correlate with the various clinical parameters associated with obesity in patients with poorly controlled type 2 diabetes after insulin therapy\(^2\). These findings show that insulin resistance can be evaluated with either HOMA-IR or KITT assessments, even when patients are receiving appropriate insulin therapy.
In the analyses separated by males and females, there was no difference between males and females in the clinical data except for HDL-C, urinary CPR and serum adiponectin. No significant difference in age, BMI, waist circumference, eVFA, systolic blood pressure, diastolic blood pressure, LDL-C, TG, HbA1c, FPG, immunoreactive insulin or ΔCPR was observed (Table 2). In men, the log-transformed KITT values correlated with eVFA and the log-transformed HOMA-IR values, but in women they correlated with BMI, waist circumference, eVFA, LDL-C, HDL-C, adiponectin and log-transformed HOMA-IR (Table 3). The women showed the correlations of KITT with more clinical data. We cannot explain what caused the difference in males and females, but we believe it is convincing that insulin tolerance is useful for evaluating insulin resistance, because...
KITT is well correlated with eVFA and log-transformed HOMA-IR in both sexes, which are considered to be the direct indices for insulin resistance.

Insulin might potentially evoke hypoglycemia, but this was rarely observed in any of the diabetic patients during the 15-min ITT in the present study. During the first 15 min of an ITT, hypoglycemia rarely occurs, and is prevented by the injection of glucose after the test. In addition, regulatory hormone concentrations have been reported to remain at basal levels throughout the test. The use of insulin sensitizers has previously been reported to be effective in type 2 diabetic patients with high insulin resistance, as estimated by an ITT. Thus, KITT values might also be useful for predicting the effectiveness of insulin sensitizers.

Insulin treatment can also stimulate the immune system to produce antibodies against the exogenous insulin used to treat patients. Therefore, insulin users might possess antibodies against insulin, and these antibodies might influence glucose homeostasis in these individuals. In such cases, insulin sensitivity cannot be precisely evaluated. Before evaluating insulin sensitivity, the level of anti-insulin antibodies circulating in the body should be determined. Additionally, patients with insulin antibody levels that might influence glucose homeostasis should be excluded from tests of insulin resistance.

In summary, the present study presented a method for measuring KITT, and confirmed the validity of this method for the evaluation of insulin sensitivity in patients with poorly controlled type 2 diabetes after they have achieved glycemic control through insulin therapy. The results also showed a close correlation between the KITT and M-values. Furthermore, KITT correlated with various clinical parameters in patients with type 2 diabetes on insulin therapy. These results suggest that KITT is a reliable and useful parameter for the evaluation of insulin sensitivity, even in patients with type 2 diabetes who undergo insulin therapy.

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