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ORIGINAL ARTICLE

Normal meal tolerance test is preferable to the glucagon stimulation test in patients with type 2 diabetes that are not in a hyperglycemic state: Comparison with the change of C-peptide immunoreactivity

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ABSTRACT

Aims/Introduction: The aim of the present study was to evaluate the properties of the glucagon stimulation test (GST) and the normal meal tolerance test (NMTT) in patients with type 2 diabetes.

Materials and Methods: We enrolled 142 patients with type 2 diabetes, and carried out a GST and a NMTT. We carried out the NMTT using a calorie-controlled meal based on an intake of 30 kcal/kg ideal bodyweight/day. We calculated the change in C-peptide immunoreactivity (ΔCPR) by subtracting fasting CPR from the CPR 6 min after the 1-mg glucagon injection (GST) or 120 min after the meal (NMTT).

Results: Mean ΔCPR for the GST was 2.0 ng/mL, and for the NMTT was 3.1 ng/mL. A total of 104 patients had greater ΔCPR in the NMTT than the GST, and the mean ΔCPR was significantly greater in the NMTT than the GST ($P < 0.05$). To exclude any influence of antidiabetic drugs, we examined 42 individuals not taking antidiabetic agents, and found the mean ΔCPR was significantly greater in the NMTT than the GST (GST 2.4 ng/mL, NMTT 4.3 ng/mL; $P < 0.05$). To consider the influence of glucose toxicity, we carried out receiver operating characteristic analyses with fasting plasma glucose and glycated hemoglobin. The optimal cut-off levels predicting GST ΔCPR to be larger than NMTT ΔCPR were fasting plasma glucose 147 mg/dL and glycated hemoglobin 9.0% (fasting plasma glucose: sensitivity 0.64, specificity 0.76, area under the curve 0.73; glycated hemoglobin: sensitivity 0.56, specificity 0.71, area under the curve 0.66).

Conclusions: The NMTT is a reliable insulin secretion test in patients with type 2 diabetes, except for those in a hyperglycemic state.

INTRODUCTION

Type 2 diabetes is a heterogeneous disease characterized by insulin resistance and defective insulin secretion[1]. The Prospective UK Diabetes Study Group reported that at the time of diabetes diagnosis, β-cell function is reduced by up to 50%, and deteriorates further regardless of therapy[2]. The glucagon stimulation test (GST) is the current standard measure of endogenous insulin secretion. However, the GST has adverse effects on patients and takes effort to administer[3]. The mixed meal tolerance test (MMTT) as an assessment of β-cell function is regarded as a more physiological test than the GST, because it evaluates typical pancreatic postprandial exposure to glucose and other nutrients, and gut and vagal hormones[4]. However, in a hyperglycemic state, meal tolerance tests (MTTs) are more affected by glucotoxicity than

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the GST. This can be a problem, because for the MMTT, patients are instructed to withhold their usual morning insulin and oral hypoglycemic agents, and ingest a standardized high-carbohydrate liquid mixed meal, which causes hyperglycemia[5]. We propose that to evaluate insulin secretion in a real-world setting, a MTT is preferable to the GST provided there is no hyperglycemia and the MTT is carried out under everyday conditions (normal meal, continued use of medications). Therefore, we carried out a normal meal tolerance test (NMTT) using a normal (calorie-controlled) meal with continued use of oral hypoglycemic agents and insulin treatments, to use for comparison with the GST. We also determined the fasting plasma glucose (FPG) and glycated hemoglobin (HbA1c) levels indicative of hyperglycemia, above which the GST is more reliable. The aim of the present study was to carry out an evaluation of the NMMT and the GST in patients with type 2 diabetes.

METHODS

The present study was cross-sectional and retrospective, and complied with the recommendations of the Declaration of Helsinki. The study was approved by the privacy policy committee of the Tottori University Hospital.

Participants

A total of 142 inpatients with type 2 diabetes participated in this study at Tottori University Hospital, Yonago, Tottori, Japan, from 2011 to 2013. Type 2 diabetes mellitus was diagnosed based on the criteria of the American Diabetes Association[6]. Patients with pancreatic disease; liver disease; pregnant; those taking diabetogenic medications, such as corticosteroids; or renal failure (serum creatinine >1.3 mg/dL) were excluded from the present study. Clinical characteristics of the participants are summarized in Table 1. Participants included 87 men and 55 women with an average age of 62 years, HbA1c of 9.4%, body mass index of 24.7 kg/m^2 and duration of diabetes of 11 years. Obesity (body mass index >30 kg/m^2) was present in 18 participants, while 55 were overweight (body mass index >25 kg/m^2). Diabetic retinopathy affected 56 participants, and diabetic nephropathy affected 51 participants. Participants were receiving the following treatments at the time of study: diet therapy alone (42 participants), oral hypoglycemic agents (OHAs) only (52 participants), insulin only (32 participants), and combined OHA and insulin (16 participants). Participants were taking the following medications: OHA (68 participants), sulfonylurea (44 participants), glinide (2 participants), biguanide (14 participants), alpha-glucosidase inhibitors (14 participants), thiazolidine (3 participants) and dipeptidyl peptidase 4 inhibitors (42 participants). A total of 48 participants were treated with insulin, 36 with bolus insulin, 40 with basal insulin and two with mixed insulin. To exclude the influence of antidiabetic drugs, we examined 42 participants that were not taking antidiabetic agents.

Table 1 | Clinical characteristics of the study participants

n	142
Sex (male/female)	80/62
Age (years)	61.5 ± 14.0
BMI (kg/m^2)	24.7 ± 4.5
Duration of diabetes mellitus (years)	11.0 ± 9.5
HbA1c, % (NGSP)	9.4 ± 2.0
Fasting plasma glucose (mg/dL)	153 ± 45
Therapy	
Diet only	42
OHA only	52
Insulin only	32
OHA + insulin	16

BMI, body mass index; HbA1c, glycated hemoglobin; NGSP, National Glycohemoglobin Standardization Program; OHA, oral hypoglycemic agents.

Study design

We carried out the GST after an overnight fast using an intravenous injection of 1 mg glucagon. C-peptide immunoreactivity (CPR) was measured at fasting before the glucagon injection (GST FCPR) and 6 min after (GST CPR-6 min) the injection[7]. The change in CPR was calculated by subtracting GST FCPR from GST CPR-6 min (GST ΔCPR). The NMTT was carried out at 08.00 hours after an overnight fast. Patients ingested a calorie-controlled breakfast prescribed as nutritional therapy according to the treatment guide for diabetes of the Japan Diabetes Society (total daily calorie intake 30 kcal/kg ideal bodyweight, single meal 25–33% of daily calorie intake with 60% of calories as carbohydrates, 20% as lipids and 20% as protein)[8,9]. For the NMTT, participants continued to take their usual OHA and insulin treatments. CPR was measured at fasting before the meal (NMTT FCPR) and 120 min after the meal (NMTT CPR-120 min). The change in CPR was calculated by subtracting NMTT FCPR from NMTT CPR-120 min (NMTT ΔCPR).

Plasma glucose was measured using the glucose oxidase method. Plasma insulin and CPR levels were measured using chemiluminescent immunoassays (human insulin and CPR chemiluminescent immunoassays kits; Kyowa Medix, Tokyo, Japan). Plasma insulin was defined as immunoreactive insulin. HbA1c was measured by high-performance liquid chromatography, and converted to National Glycohemoglobin Standardization Program values using the following equation: National Glycohemoglobin Standardization Program (%) = 1.02 × Japan Diabetes Society (%) + 0.25%[10].

Statistical analysis

Data are presented as mean ± SEM. Differences in mean values were determined using unpaired *t*-tests, and considered statistically significant at $P < 0.05$. In the hyperglycemic state, the MTT ΔCPR is more affected by glucotoxicity than the GST ΔCPR. Therefore, we determined cut-off values with a receiver

operating characteristic (ROC) analysis of FPG and HbA1c. The sensitivity of the FPG and HbA1c cut-off points was defined as the ability of FPG or HbA1c to identify the GST ΔCPR as larger than the NMTT ΔCPR. The specificity was defined as the ability of FPG or HbA1c to identify the GST ΔCPR as smaller than the NMTT ΔCPR. To evaluate the abilities of FPG and HbA1c to detect the reactivity of ΔCPR, we plotted ROC curves. Diagnostic properties of the cut-off levels of FPG and HbA1c were defined by maximizing the sensitivity and specificity to identify the GST ΔCPR as larger than the NMTT ΔCPR. A ROC curve is a graph of sensitivity vs (1-specificity) for various cut-off definitions of a positive diagnostic test result. The optimal cut-off points were obtained using the Youden Index (maximum [sensitivity + specificity-1]), and the point on the ROC curve closest to (0,1) was calculated as the minimum value of the square root of ([1-sensitivity]2 + [1-specificity]2)[11,12]. Greater accuracy is reflected by a larger Youden Index and a smaller distance to (0, 1). SPSS 15.0 software (SPSS, Chicago, Illinois, USA) was used for analysis.

RESULTS

For the GST, the mean plasma glucose at fasting and 6 min after glucagon injection was 146 ± 38 and 161 ± 37 mg/dL, respectively. The mean GST FCPR and the GST CPR-6 min were 2.1 ± 1.1 and 4.1 ± 2.1 ng/mL, respectively, and the mean GST ΔCPR was 2.0 ± 1.3 ng/mL. For the NMTT, the mean plasma glucose at fasting and 120 min post-meal was 154 ± 45 and 255 ± 66 mg/dL, respectively. The mean NMTT FCPR and NMTT CPR-120 min were 2.1 ± 1.1 and 5.3 ± 2.6 ng/mL, respectively, and the mean NMTT ΔCPR was 3.1 ± 1.9 ng/mL (Table 2). Of the 142 participants, 104 showed a greater ΔCPR in the NMTT than the GST, and the mean NMTT ΔCPR was significantly greater than the mean GST ΔCPR ($P < 0.05$; Figure 1). To exclude any influence of antidiabetic drugs, we examined 42 participants that were not using antidiabetic agents. The clinical characteristics of these participants are summarized in Table 3. For participants not using antidiabetic drugs, the mean GST ΔCPR was 2.4 ng/mL and the mean NMTT ΔCPR was 4.3 ng/mL, and NMTT ΔCPR was significantly greater than GST ΔCPR ($P < 0.05$; Table 4).

To consider the influence of glucose toxicity, we carried out ROC analyses with FPG and HbA1c. The optimal cut-off levels of FPG and HbA1c to identify the GST ΔCPR as larger than the NMTT ΔCPR were 147 mg/dL and 9.0%, respectively. In FPG 147 mg/dL, sensitivity was 0.64 and specificity was 0.76, and area under ROC curve (AUC) was 0.73, representing moderate accuracy. Similarly, in HbA1c 9.0%, sensitivity was 0.56 and specificity was 0.71, and AUC was 0.66, representing low accuracy (Figure 2).

DISCUSSION

In the present study, the NMTT ΔCPR was greater than the GST ΔCPR. This result agrees with a previous study on patients with type 1 diabetes that found the MMTT to be superior to the GST when carried out under standardized conditions[3]. This is probably because the MMTT causes an incretin

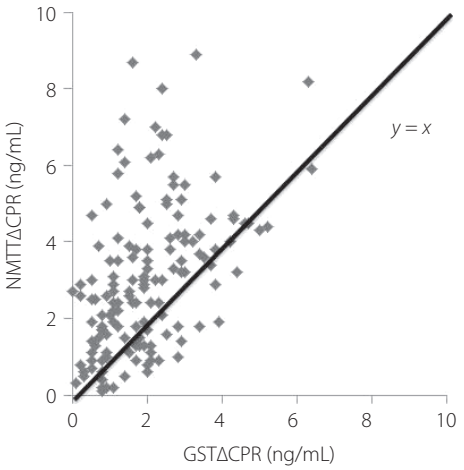

Figure 1 | Results of C-peptide immunoreactivity (CPR) 6 min after glucagon injection – fasting CPR (GSTΔCPR) and CPR from CPR 120 min after meal – fasting CPR (NMTTΔCPR). The line on the graph expresses $y = x$. As for the participant above the line, NMTTΔCPR is higher than GSTΔCPR.

Table 2 | Results of glucagon stimulation test and the normal meal tolerance test

	GST	NMTT	*P*
Fasting PG (mg/dL)	146 ± 38	154 ± 45	<0.05
After load PG (mg/dL)	161 ± 37	255 ± 66	<0.05
Fasting CPR (ng/mL)	2.1 ± 1.1	2.1 ± 1.1	0.11
After load CPR (ng/mL)	4.1 ± 2.1	5.3 ± 2.6	<0.05
ΔCPR (mg/dL)	2.0 ± 1.3	3.1 ± 1.9	<0.05

Data represent mean ± SD. After load, 6 min after glucagon injection in the glucagon stimulation test or 120 min after a meal in the normal meal tolerance test; CPR, C-peptide immunoreactivity; GST, glucagon stimulation test; PG, plasma glucose. ΔCPR = after load CPR – fasting CPR.

Table 3 | Clinical characteristics of participants not using antidiabetic drugs

n	42
Sex (male/female)	26/16
Age (years)	61.8 ± 14.1
BMI (kg/m^2)	24.7 ± 4.5
Duration of diabetes mellitus (years)	6.6 ± 7.8
HbA1c, % (NGSP)	8.7 ± 1.7
Fasting plasma glucose (mg/dL)	146 ± 34

Data represent mean ± SD. BMI, body mass index; HbA1c, glycated hemoglobin; NGSP, National Glycohemoglobin Standardization Program.

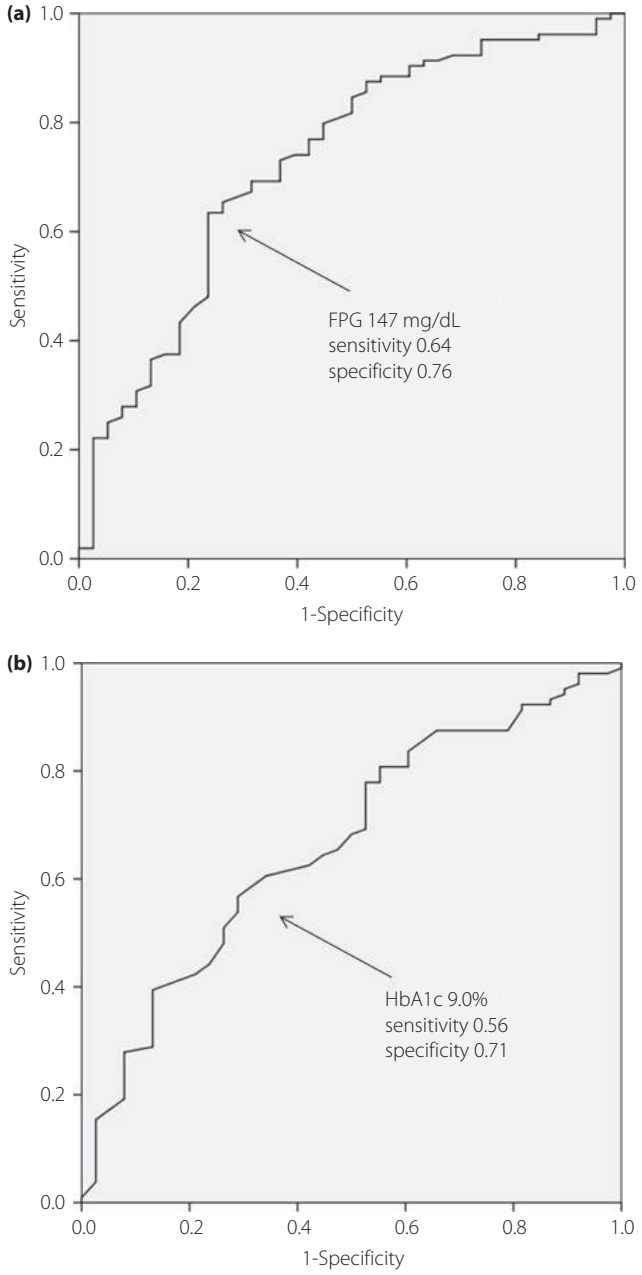

Figure 2 | Change in C-peptide immunoreactivity (ΔCPR) during the (a) glucagon stimulation test (GST) and (b) normal meal tolerance test (NMTT). Receiver operating characteristic curves for change in C-peptide immunoreactivity (ΔCPR) used to predict the cut-off points when ΔCPR of the glucagon stimulation test (GST) is larger than that of the normal meal tolerance test (NMTT). Sensitivity is plotted as a function of (1-specificity). Points on the curve representing optimal fasting plasma glucose (FPG) and glycated hemoglobin (HbA1c) are marked with an arrow.

effect whereby the oral glucose stimulus elicits greater insulin secretion than a similar intravenous stimulus, through gastric inhibitory polypeptide and glucagon-like peptide 1. The MMTT is thus the gold standard measure of endogenous insulin

Table 4 | Results of the glucagon stimulation test and normal meal tolerance test in participants not using antidiabetic drugs

	GST	NMTT	*P*
Fasting PG (mg/dL)	141 ± 31	146 ± 34	0.06
After load PG (mg/dL)	157 ± 32	247 ± 61	<0.05
Fasting CPR (ng/mL)	2.3 ± 0.9	2.3 ± 0.9	0.43
After load CPR (ng/mL)	4.7 ± 2.0	6.6 ± 2.4	<0.05
ΔCPR (mg/dL)	2.4 ± 1.3	4.3 ± 1.8	<0.05

Data represent mean ± SD. After load, 6 min after glucagon injection in the glucagon stimulation test or 120 min after a meal in the normal meal tolerance test; CPR, C-peptide immunoreactivity; PG, plasma glucose. ΔCPR = after load CPR − fasting CPR.

secretion for patients with type 1 or type 2 diabetes[4]. However, while the full MMTT is used in research, it is rarely carried out in routine clinical practice because of the intensity of sampling required (samples are required every 30 min for 2 h to allow measurement of AUC and peak CPR)[13]. Furthermore, the MMTT has several problems as follows. First, the mixed meal is liquid and composition is different from the normal food, so the stimulation of insulin secretion might be different from the normal food. Second, fixed-calorie test meals provide weak stimulation for taller patients and strong stimulation for shorter patients. Instead, we propose that calorie-controlled meals would be preferable to fixed-calorie test meals for assessment of insulin secretion ability in a real-world setting. Here, we carried out a NMTT using calorie-controlled meals adjusted for ideal bodyweight, and measured CPR just twice: at fasting and 2 h after the meal. This NMTT is simple, making it more practical for use in a clinical setting than the full MMTT or the GST.

In the chronic hyperglycemia state, glucose toxicity deteriorates meal-induced insulin secretion[14]. Funakoshi *et al.*[15] reported that chronic high blood glucose shown by high HbA1c levels might impair endogenous insulin secretion after a meal load, but has little effect on endogenous insulin secretion after a glucagon load. We also found that the NMTT ΔCPR was more affected by glucose toxicity than the GST ΔCPR. Therefore, we carried out ROC analyses to determine the cut-off values of FPG and HbA1c. The cut-off values of FPG and HbA1c at which GST ΔCPR was larger than NMTT ΔCPR were 147 mg/dL and 9.0%, respectively. Thus, in the hyperglycemic state, especially when FPG is 147 mg/dL and HbA1c is ≥9.0%, the NMTT ΔCPR might be an underestimate, and GST should instead be carried out to assess endogenous insulin secretion.

The present study had several limitations. First, the sample size of the study was small, and we did not measure incretins. We propose that the MTT ΔCPR was larger than the GST ΔCPR because of the incretin effect, and that the incretin effects were diminished in the hyperglycemic state. We hope to measure incretins in future larger studies. Second, to assess insulin secretion ability in a real-world setting and reduce the hyperglycemia caused by the MTT, we continued the

participants' use of their antidiabetic drugs, including OHAs and insulin treatment. Sulfonylurea, glinide and dipeptidyl peptidase 4 inhibitors encourage insulin secretion, so might have affected CPR response in the MTT. A prior study of patients with type 1 or type 2 diabetes receiving insulin treatment showed that there was a 20% reduction in peak CPR value during a MMTT when bolus insulin was given compared with no bolus insulin given[16]. Another study reported that reducing chronic hyperglycemia by basal insulin therapy enhanced endogenous β-cell function after a MMTT[17]. These reports suggest that insulin therapy affected CPR response in the MTT. However, in the present study, the same results were obtained from participants not using OHAs or insulin treatment, as from all participants. Therefore, we propose that the NMTT is useful in evaluating endogenous insulin secretion ability even for patients taking antidiabetic medications. Giving antidiabetic drugs to patients undergoing the NMTT makes little difference to its ability to detect endogenous insulin secretion. Third, we assessed fasting and 2-h CPR during the NMTT. Greenbaum *et al.*[3] reported that in the MMTT, CPR usually peaks around 90 min in patients with type 1 diabetes, and 90 min CPR has been shown to be related to improved clinical outcomes[18]. However, in the present study, we aimed to identify a method for assessing insulin secretion ability that is easy to use during daily clinical work. We assess postprandial plasma glucose at 2 h in daily clinical work, so chose to assess postprandial CPR at the same time to streamline sampling efforts.

In conclusion, the NMTT is valuable as an insulin secretion test in patients with type 2 diabetes, except under conditions of hyperglycemia. In the hyperglycemic state, especially where FPG is 147 mg/dL or HbA1c is ≥9.0%, the GST should be used instead. Thus, the question of whether to use the GST or the NMTT for measurement of insulin secretion can be answered using levels of FPG or HbA1c.

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DISCLOSURE

The authors declare no conflict of interest.

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