Research Article

Predictive factors of efficacy of combination therapy with basal insulin and liraglutide in type 2 diabetes when switched from longstanding basal-bolus insulin: Association between the responses of β- and α-cells to GLP-1 stimulation and the glycaemic control at 6 months after switching therapy

Ichiro Horie, Ai Haraguchi, Ayaka Sako, Junya Akeshima, Tetsuro Niri, Riyoko Shigeno, Ayako Ito, Aya Nozaki, Shoko Natsuda, Satoru Akazawa, Yoshitaka Mori, Takao Ando, Atsushi Kawakami, Norio Abiru

Department of Endocrinology and Metabolism, Nagasaki University Hospital, Nagasaki, Japan

Correspondence to: Ichiro Horie, M.D., Ph.D. Department of Endocrinology and Metabolism, Nagasaki University Hospital, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.

Tel.: +81-95-819-7262; Fax: +81-95-849-7270. E-mail: holy197741@me.com

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ABSTRACT

Aims: To evaluate the glycaemic control of combination therapy with basal insulin and liraglutide, and to explore the factors predictive of efficacy in patients with type 2 diabetes when switched from longstanding basal-bolus insulin therapy.

Methods: We studied 41 patients who switched from basal-bolus insulin therapy of more than 3 years to basal insulin/liraglutide combination therapy. Glycaemic control was evaluated at 6 months after switching therapy and used to subdivide the patients into good-responders (HbA1c <7.0% or 1.0% decrease) and poor-responders (the rest of participants). To evaluate the glucose-dependent insulin/glucagon responses without/with liraglutide, a 75-g oral glucose tolerance test (OGTT) was performed twice, before (1^{st}-OGTT) and 2-days after (2^{nd}-OGTT) liraglutide administration.

Results: Twenty-eight patients (68.3%) were identified as good-responders. No differences were found in baseline characteristics including insulin/glucagon responses during 1^{st}-OGTT between the groups. 2^{nd}-OGTT revealed that paradoxical hyperglucagonemia were significantly improved in both groups, but significant increases in insulin secretory response were observed only in good-responders. Logistic regression analyses revealed that the improvement of the insulin-response during 2^{nd}-OGTT compared to that during 1^{st}-OGTT is associated with the good-responder.

Conclusions: Enhancement of glucose-dependent insulin-response under liraglutide administration is a potential predictor of long-term glycaemic control after switching the therapies.

Keywords: liraglutide; GLP-1; basal; bolus; proinsulin; glucagon

Abbreviations: OADs, oral anti-diabetic drugs; GLP-1, glucagon-like peptide-1; GLP-1RA, GLP-1 receptor agonist; BBT, basal-bolus insulin therapy; BGT, basal insulin and GLP-1RA combination therapy; OGTT, oral glucose tolerance test; ELISA, enzyme-linked immunosorbent assay; $\Delta Glucose_{120min}$, the increases in plasma glucose levels from 0 to 120 min during OGTT; $\Delta Insulin_{120min}$, the increases in serum insulin levels from 0 to 120 min during OGTT; $\Delta Glucagon_{120min}$, the suppressions of plasma glucagon levels from 0 to 120 min during OGTT, ROC, receiver operating characteristic.

1. INTRODUCTION

The typical clinical course of type 2 diabetes is characterized by progressive deterioration of the pancreatic β-cells that synthesize and secret insulin [1], resulting in worsening glycaemic control and sequential addition of anti-diabetic agents, including insulin, over time. The basal-bolus insulin regimen, in which patients take basal insulin once a day and rapid-acting insulin before each meal, is typically the final step in the therapeutic progression [2]. Even with intensive basal-bolus insulin therapy, however, in most cases it is difficult to achieve excellent glycaemic control clinically due to the high risks of hypoglycaemia and weight gain by inadequate insulin dosage.

The glucagon-like peptide-1 (GLP-1) receptor agonist has recently garnered attention as a breakthrough drug for patients with type 2 diabetes. The GLP-1 receptor agonist (GLP-1RA) has several potential benefits because it enhances insulin secretion glucose-dependently and concurrently ameliorates insulin resistance through weight reduction [3]. It has also been shown that GLP-1RA ameliorates post-prandial hyperglycaemia by suppressing glucagon secretion and delaying gastric emptying [3]. Eng and colleagues reported their findings from a systematic review and meta-analysis of evidence about the combination of basal insulin and GLP-1RA in type 2 diabetes. Compared with basal-bolus insulin therapy, treatment with GLP-1RA plus basal insulin

gave a clinically insignificant reduction in HbA1c, but with a lower relative risk of hypoglycaemia and a reduction in body weight [4]. As a result, this combination therapy is becoming increasingly common in the treatment of type 2 diabetes [5, 6]. GLP-1 receptor agonists are subdivided into short-acting compounds and long-acting compounds such as liraglutide. The long-acting GLP-1 receptor agonists have a lower incidence of adverse effects such as nausea and vomiting [7]. It is possible that a regimen combining a once-a-day injection of basal insulin with long-acting GLP-1RA would be an effective alternative to a basal-bolus insulin regimen with less gastrointestinal discomfort and less treatment burden.

Several pilot studies in Japan have retrospectively shown that the response to GLP-1RA is associated with fasting or postprandial serum C-peptide levels or the C-peptide response to glucagon-challenge test in type 2 diabetes patients at the initiation of GLP-1RA without any concomitant insulin regimen [8-10]. These facts suggest that the individual's responses to mono-injectable therapy of GLP-1RA rely on the residual β-cell function and that GLP-1RA might be effective to some degree for type 2 diabetes patients with reduced β-cell function when combined with basal insulin. Moreover, Germain and colleagues recently reported that a higher insulin sensitivity calculated by insulin tolerance test was another predictive marker for a mono-injectable therapy of liraglutide with OADs.

[11].

Very recently, Usui and colleagues demonstrated a retrospective study that the residual β-cell function indicated by the fasting C-peptide index was a predictive marker for the achievement of HbA1c <7.0% by combination therapy with basal insulin and liraglutide 1 year after switching from therapy with basal-bolus or basal insulin with OADs [12]. However, it remains unclear what kind of patients would most benefit from such a switch in therapy to a combination with basal insulin and GLP-1RA, especially among those who had received longstanding basal-bolus insulin therapy, that is the final step in the therapeutic progression in type 2 diabetes.

In this study, we prospectively examined what determines the long-term efficacy of replacing bolus insulin with liraglutide in patients with type 2 diabetes treated with longstanding basal-bolus insulin therapy from the standpoint of the responses of β- and α-cells to GLP-1 stimulation.

2. MATERIALS AND METHODS

2.1. Participants

Eligible type 2 diabetic patients were Japanese adults over 20 years of age who had

been under treatment with basal-bolus insulin therapy (herein BBT) for more than 3 years prior to being enrolled in this study. None of the participants had previously been treated with GLP-1RA. Patients were excluded if they had type 1 diabetes, severe renal disorder with an estimated glomerular filtration rate < 20 mL/min/1.73m^2 and/or current dialysis, liver disease, chronic pancreatitis, history of gastrointestinal surgery or pancreatomy, alcohol abuse, diabetogenic medications including steroids, malignancy or pregnancy.

2.2 Study design

This was a single-centre, open-label, prospective cohort study in patients with type 2 diabetes at Nagasaki University Hospital from April 2015 to September 2017 (UMIN-CTR, UMIN000021693). The study protocol is shown in Fig. 1.

The participants were admitted to Nagasaki University Hospital and achieved a good level of glycaemic control (fasting glucose levels, 80-120 mg/dL) by using intensified basal-bolus insulin while discontinuing all OADs, excluding metformin and pioglitazone. Liraglutide was started at 0.3 mg before breakfast and increased to 0.6 mg on the following day. The 75-g oral glucose tolerance test (OGTT) was used to estimate β- and α-cell functions before starting liraglutide (1st-OGTT). The OGTT was re-performed two days after starting liraglutide (2nd-OGTT) to evaluate the responses of β- and α-cells

by GLP-1 stimulation. Liraglutide was injected 30 minutes prior to starting the 2nd-OGTT. Both OGTTs were carried out under the condition of overnight fasting. Basal insulin and OADs including metformin and pioglitazone were held until the preceding day in each OGTT.

After the 2nd-OGTT, the patients were withdrawn from bolus insulin and switched to the simultaneous injections of basal insulin and GLP-1RA (liraglutide) combination therapy (herein BGT) in the morning. Clinicians were permitted to change the doses of basal insulin, liraglutide and metformin in the outpatient setting to keep the fasting glucose levels under 120 mg/dL. The maximum doses of liraglutide and metformin permitted in Japan were 0.9 mg and 2250 mg per day, respectively. We discontinued liraglutide and excluded the participants from the study if we observed potential adverse events of liraglutide, such as severe hypoglycaemia requiring hospitalization, unbearable nausea or vomiting, stroke, myocardial infarction, malignancy and any other diseases that needed hospitalization. The glycaemic status of patients was evaluated at 6 months after switching therapy and the patients were sub-divided into good-responders, defined as those with HbA1c <7.0% or a decrease in HbA1c of more than 1.0%, and poor-responders, defined as those meeting neither criterion.

The patients were all encouraged to practice appropriate diet and exercise and

educated in regard to this practice throughout the study period. As a nutrition therapy, the participants were instructed to intake a 25-30 kcal/kg/day of their ideal body weight depending on the amount of activity of daily living, comprised of carbohydrates at 50-60% of total calories. Written informed consent was obtained from all participants. The study was approved by the ethical committee of Nagasaki University Hospital (approval no. 15012676) and carried out in accordance with the declaration of Helsinki.

2.3. Laboratory measurements

As described above in the study design and in Fig 1, OGTTs were carried out twice (1^{st}- and 2^{nd}-OGTT) using a 75-g glucose formulation, Trelan-G75 (AY Pharma, Tokyo, Japan). The levels of plasma glucose (mg/dL), serum insulin (μU/mL), serum C-peptide (ng/mL) and plasma glucagon (pg/mL) were measured at fasting (0 min) and at 30, 60, 120 min after ingestion of glucose load on each OGTT. The fasting levels of serum proinsulin (pmol/L) were also measured on both 1^{st}- and 2^{nd}-OGTT.

The levels of serum insulin and C-peptide were measured by using an ECLusys kit (Roche, Basel, Switzerland). The insulin assay cannot detect any insulin analogues but measure human insulin specifically. Blood samplings for plasma glucagon were performed using BD P800 tubes (BD, Franklin Lakes, NJ, USA). Plasma glucagon was measured as

previously described [13], by using a recently produced sandwich enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Uppsala, Sweden) with almost no cross-reactivities against other glucagon-related peptides [14, 15]. Proinsulin was measured by using a sandwich ELISA kit (Mercodia, Uppsala, Sweden). Other laboratory measurements including plasma glucose were measured by standard assays.

C-peptide index was calculated as 100 × C-peptide (ng/mL)/glucose (mg/dL) as an estimated residual β-cell function. The values of the changes from baseline (0 min) to 120 min during the OGTTs in the levels of glucose, insulin, C-peptide and glucagon were indicated as $\Delta Glucose_{120min}$, $\Delta Insulin_{120min}$, $\Delta C\text{-}peptide_{120min}$ and $\Delta Glucagon_{120min}$, respectively. Three-day means of blood glucose levels just before discharge from the hospital were shown as the profile of mean blood glucose levels just after switching from BBT to BGT.

2.4. Statistical analysis

Repeated-measures analysis of variance was used to test differences between good-responders and poor-responders. A paired t-test was used to test differences in each time point between 1^{st}-OGTT and 2^{nd}-OGTT in a single group. Logistic regression analyses were performed to determine the long-term efficacy of the BGT at 6 months after

switching from BBT in the patients. Receiver operating characteristic (ROC) curves were used to calculate the accuracy of the parameters determined significantly as predictive markers of a good-responder at 6 months after switching from BBT to BGT. Statistical analysis was carried out using JMP Pro version 11.2 (SAS Institute, Cary, NC, USA). P-values less than 0.05 were considered significant.

3. RESULTS

A total of 49 patients agreed to participate in the study. Among them, 8 participants were excluded: in 4 of these cases type 1 diabetes could not be ruled out through the detection of positive anti-islet autoantibodies in the serum, and in 4 of them some portion of the required data, including results of anti-islet autoantibodies or some results of insulin and glucagon levels during the OGTTs, were not available. We thus enrolled 41 patients in the study, and all of them were able to continue BGT for more than 6 months of the study period without any adverse events. Among the 41 patients, 28 (68.3%) were classified as good-responders and 13 (31.7%) as poor-responders at 6 months after switching therapy. The baseline characteristics of the patients are shown in Table 1. The daily dose of basal insulin was significantly lower in good-responders than in

poor-responders. The other baseline parameters, including age, height, weight, body mass index (BMI), duration of diabetes, duration of insulin treatment prior to the study, duration of BBT prior to the study, degrees of glycaemic control and diabetic complications, residual β-cell function estimated by the C-peptide index at fasting and 2h after a 75-g glucose load, total daily dose of bolus insulin and use of OADs were not significantly different between good- and poor-responders. There were no significant differences in three-day means of blood glucose levels at each pre-prandial and bedtime just after switching from BBT to BGT between good- and poor-responders. (Fig. 2a).

HbA1c levels in good-responders were significantly decreased compared to baseline as early as 3 months after switching therapy and maintained until 6 months, while those in poor-responders were significantly increased compared to baseline at 6 months after switching therapy (Fig. 2b). Severe hypoglycaemia requiring medical treatment was not observed in any of the participants during the study period even though more than half (56.1%) of the good-responders achieved HbA1c <7.0% at 6 months after switching therapy.

Body weights were similarly decreased in both groups after switching therapy (Fig. 2c). The dosages of liraglutide were increased after switching therapy in both groups while the dosage needed in good-responders was significantly less than that needed in

poor-responders at 3 months (Fig. 2d). More basal insulin was required in poor-responders than in good-responders on admission and at 3 and 6 months after switching therapy (Fig. 2e). In some patients, metformin usage was increased during hospitalization, but there were no significant differences in the use of metformin after discharge in each group (Fig. 2f).

Under baseline conditions during 1st-OGTT, not only plasma glucose but also insulin/glucagon responses to glucose load were similar between good-responders and poor-responders (Fig. 3a-d, Supplementary Fig. 1). Under liraglutide administration during 2nd-OGTT, plasma glucose excursion was significantly decreased compared to that during 1st-OGTT in both groups (Fig. 3a). The increases in plasma glucose levels from 0 to 120 min ($\Delta Glucose_{120min}$) were significantly suppressed after liraglutide administration in both groups (Fig. 3e). Insulin responses to glucose load during 2nd-OGTT were significantly increased compared to those during 1st-OGTT in good-responders but not in poor-responders (Fig. 3b), although fasting serum insulin concentrations were significantly elevated just after starting liraglutide in both groups. The changes in the serum levels of insulin from 0 to 120 min ($\Delta Insulin_{120min}$) during 2nd-OGTT were significantly increased compared to those during 1st-OGTT in good-responders but not in poor-responders (Fig. 3f). The data of increased response of serum C-peptide levels during

2nd-OGTT compared to those during 1st-OGTT in good-responders but not in poor-responders (Fig. 3c, g) were similar to those of serum insulin levels (Fig. 3b, f). Plasma glucagon concentrations at each time-point of OGTT were not significantly different between the 1st-OGTT and 2nd-OGTT in either group (Fig. 3d). However, the suppressions of glucagon secretion from 0 to 120 min ($\Delta Glucagon_{120min}$) during 2nd-OGTT were significantly larger than those during 1st-OGTT in each group (Fig. 3h).

We also studied the molar ratios of insulin to glucagon and of proinsulin to insulin in the fasting sera derived from 1st- and 2nd-OGTT samples. Before liraglutide administration, there were no differences in these ratios between good- and poor-responders. Interestingly, a significant increase in the fasting insulin/glucagon ratio and decrease in the fasting proinsulin/insulin ratio 2 days after liraglutide administration (2nd-OGTT) compared to baseline (1st-OGTT) were observed in good-responders but not in poor-responders (Fig. 3i, j).

In order to identify predictive factors for good-responders, we performed logistic regression analyses of clinical characteristics or insulin/glucagon responses obtained from each of the OGTTs and from the data of the 1st-OGTT subtracted from the data of the 2nd-OGTT (Table 2). No factors were found to be significantly predictive of a good responder among either clinical characteristics or the results of OGTT before liraglutide

administrations (1st-OGTT). From the subtraction data, only "Increase in $\Delta Insulin_{120min}$," which represents the value derived from subtracting $\Delta Insulin_{120min}$ in the 1st-OGTT from that in the 2nd-OGTT, was significantly associated with good glycaemic control at 6 months after switching therapy. The ROC analysis showed that the cut-off value of "Increase in $\Delta Insulin_{120min}$" to predict a good-responder at 6 months after switching the therapy was 1.8 μU/mL (area under the curve 0.753; 95% confidence interval 0.537-0.889) with 93% sensitivity and 62% specificity (Fig. 3k).

4. DISCUSSION

Here we found that more than two thirds of type 2 diabetic patients with decreased insulin secretory capacity who had needed intensive basal-bolus therapy showed good glycaemic controls when bolus insulin was replaced with liraglutide. It has been shown that the efficacy of switching injectable therapy from insulin to liraglutide in the patients with type 2 diabetes depends on the sustained endogenous insulin-secreting capacity [9, 10, 16, 17]. We previously demonstrated that sulfonylurea use before liraglutide treatment was associated with poor glycaemic response to mono-injectable therapy of liraglutide among patients with type 2 diabetes [18]. It has been shown that

patients with diabetes using sulfonylurea have reduced endogenous insulin secretion [19]. Because most of the patients in our current study had required intensive basal-bolus insulin therapy to maintain their glucose levels, their baseline capacity of insulin secretion seemed to be decreased overall, as shown in Fig. 3. Liraglutide might be effective even in patients with type 2 diabetes with reduced residual β-cell functions when used with basal insulin, due to the compensation of insulin action. However, not all the patients exhibited an improvement of glycaemic control upon switching from basal-bolus therapy to combination therapy with basal insulin/liraglutide. One third of cases exhibited worse glycaemic control, although the body weights were reduced after switching therapy. It is clinically important to predict the efficacy of switching therapy in patients with type 2 diabetes. We therefore explored the predictive factors of the efficacy from the point of view of clinical features, including the responses of β- and α-cells to glucose load, not only at baseline (1^{st}-OGTT) but also under liraglutide administration (2^{nd}-OGTT).

First, we found no significant differences in baseline parameters except the dosage of basal insulin between good-responders and poor-responders (Table 1). In addition, no significant differences in the β- and α-cell functions were observed between the two groups in the 1^{st}-OGTT (Supplementary Fig. 1). These facts suggest that it is difficult to predict the long-term glycaemic control after switching therapy. Usui et al.

recently reported that the residual β-cell function indicated as the fasting C-peptide index > 1.103 was a predictive marker for the achievement of HbA1c <7.0% by combination therapy with basal insulin and liraglutide at 1 year after switching from therapy with basal-bolus or basal insulin with OADs [12]. Our data on the baseline C-peptide indexes and endogenous insulin responses observed in 1st-OGTT in the good-responders were not significantly different from the data in the poor-responders (Table 1, Supplementary Fig. 1). ROC analysis for baseline C-peptide index to predict a good-responder of our study did not reach a significant level (Supplementary Fig. 2). If Usui's value of 1.103 could have been applied in our study for the cut-off of C-peptide index for the achievement of HbA1c <7.0% at 6 months after switching therapy, it would have been calculated at 43% for sensitivity, 78% for specificity, 71% for positive-predictive value, and 52% for negative-predictive value. Unfortunately, we could not follow up the glycaemic controls of the participants until 1 year after switching therapy. Furthermore, the participants of Usui's study included patients with relatively preserved β-cell function who did not need bolus insulin prior to enrolling into the study. It is probable that the residual β-cell functions of our patients were decreased more than those of Usui's study. The discrepancy about C-peptide index between Usui's study and our study might have been caused by some degree of difference in the study designs and the baseline characteristics of eligible

subjects.

Second, we found that the responses of insulin secretion to oral glucose load were significantly enhanced just after liraglutide administration (2^{nd}-OGTT) in good-responders but not in poor-responders. These facts might suggest that liraglutide could improve β-cell function even in the patients with long-standing disease and minimal baseline capacity of insulin secretion. Our logistic regression analyses demonstrated that "Increase in $\Delta Insulin_{120min}$", which indicates the enhancement of insulin response to glucose load under liraglutide administration, was the only marker associated with good glycaemic control after switching therapy (Table 2). Especially, ROC analyses estimated the cut-off value of "Increase in $\Delta Insulin_{120min}$" to predict good response 6 months after switching therapy was 1.8μU/mL with 84% for positive-predictive value and 73% for negative-predictive value (Fig. 3k). Although this threshold of "Increase in $\Delta Insulin_{120min}$" could not provide clinicians with a perfect information to predict the response, these findings suggest that the dual evaluation of the glucose-dependent insulin responses before and just after liraglutide administration could be a predictive approach to determine whether liraglutide can replace bolus insulin in patients who had longstanding basal-bolus therapy. Eventually, it could avoid a long-term "try and see" prescription pattern.

To investigate the mechanisms of the association between the enhancement of

insulin response under liraglutide administration and glycaemic control 6 months after switching therapy, we evaluated the fasting ratio of proinsulin/insulin, which is reflected in the degree of insulin processing and the secretion of more immature insulin granules. Increases in the ratio are seen in patients with recent-onset type 1 diabetes [20] and those with type 2 diabetes [21], and are already apparent in the stage of impaired glucose tolerance [22]. These are generally thought to be the consequence of chronic β-cell stimulation in conditions with β-cell loss and to reflect a primary reduction of insulin secretory capacity [23, 24]. In this study, we observed that the fasting proinsulin/insulin ratio was significantly decreased 2 days after liraglutide administration compared to that before administration in good-responders but not in poor-responders (Fig. 3j). This might reflect that the recovery from β-cell exhaustion seen after liraglutide administration was more remarkable in good-responders than in poor-responders.

Of note, the short-term (2-day) administration of liraglutide improved the glucose excursion during OGTT in poor-responders without an enhancement of insulin response (right panel of Fig. 3a, b). A possible mechanism is the effect of GLP-1 on the suppression of glucagon secretion or that on the delay of gastric emptying. We observed paradoxical increases in glucagon secretion after ingestion of glucose, or so-called paradoxical hyperglucagonemia [25], before liraglutide administration in both good- and

poor-responders (left panel of Supplementary Fig. 1d). A mere 2-day administration of liraglutide improved the paradoxical hyperglucagonemia and the glucagon suppression during the 120 minutes of OGTT ($\Delta Glucagon_{120min}$) to a comparable degree between the two groups (right panel of Supplementary Fig. 1d). Logistic regression analyses also demonstrated that there was no association between the improvement of glucagon suppression and a good treatment response, as shown in Table 2. These findings suggest that the improvement of the glucose excursion during OGTT in poor-responders might have been affected by the effect of GLP-1 on the delaying gastric emptying rather than the effect of GLP-1 on the suppression of glucagon secretion. It has been shown that the slowing of gastric emptying by long-acting GLP-1RAs such as liraglutide becomes attenuated over time, while the effect by short-acting GLP-1RAs is maintained [26]. These phenomena might be partially associated with the worsening of glycaemic control in the poor-responders after switching therapy over time (Fig. 2b).

The current study had several limitations. First, the sample size was small because the study was administered in a single centre. Second, we could not show the efficacy more than 6 months after switching therapy because we terminated the study at 6 months. Third, we could not evaluate the insulin resistance of the participants on the baseline. Germain et al. recently reported that the insulin resistance evaluated by insulin

tolerance test was useful to predict the efficacy of mono-injectable therapy with liraglutide in patients with type 2 diabetes who had been treated with OADs. We did not evaluate the insulin resistance of the patients by insulin tolerance test. Since we carried out 1st-OGTT before administration of liraglutide while under basal insulin, metformin and pioglitazone until the preceding day of the OGTT, the homeostatic model assessment for insulin resistance [27] and Matsuda index [28] were not able to estimate the baseline insulin resistance of the patients. Fourth, there was no precise rule for the dose adjustments of basal insulin by the clinicians, except that the fasting glucose level should be maintained below a target of 120 mg/dL. In an outpatient setting, especially for the poor-responders, it might be insufficient to increase the use of basal insulin to maintain lower fasting glucose levels. It has been demonstrated that the effects of GLP-1 on the secretion of insulin and glucagon are decreased in patients with poorly controlled fasting glucose levels [29]. The incretin effect had already declined when the glucose concentrations exceeded the upper limit of normal. This was thought to be a consequence of a hyperglycaemia-induced downregulation of GLP-1 receptor expression [30, 31]. Fifth, we could not evaluate compliance to the diet therapy and exercise education. It might be a bias between good-responders and poor-responders. Sixth, the maximum dose of liraglutide permitted in Japan is 0.9 mg per day. Therefore, our approach would not be sufficient for patients in

other countries where liraglutide of up to 1.8 mg per day has been approved.

In summary, we demonstrated that liraglutide can replace bolus insulin in more than two thirds of Japanese patients with type 2 diabetes with reduced β-cell function who have required longstanding basal-bolus insulin. Before switching to the combination of basal insulin/liraglutide, it may be hard to predict the long-term glycaemic responses of the combination therapy, even if the baseline β- and α-cell functions are evaluated precisely. However, dual evaluation of the insulin responses to glucose load before and just after liraglutide administration could be valuable to predict long-term glycaemic control when replacing bolus insulin with liraglutide in patients treated with longstanding basal-bolus insulin.

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DISCLOSURE

The authors declare no conflict of interest.

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FIGURE LEGENDS

Figure 1. The study design.

(a) A 75-g OGTT was performed before (1^{st}-OGTT) and 2 days after starting liraglutide (2^{nd}-OGTT) to evaluate the glucose-dependent responses of β- and α-cells by the GLP-1

stimulation. *The maximum dose of liraglutide permitted in Japan is 0.9 mg per day.

(b) Blood samplings were performed at fasting (0 min), and 30, 60, 120 min after ingestion of glucose in each OGTT. Liraglutide was injected 30 minutes prior to starting the 2nd-OGTT. Basal insulin and oral anti-diabetic agents including metformin and pioglitazone were held until the preceding day in each OGTT.

Figure 2. Comparisons of the outcomes and treatments between good-responders (n=28) and poor-responders (n=13).

(a) Three-day means of blood glucose levels just before discharge from the hospital in good-responders (white bar) vs. poor-responders (slashed bar). BB, before breakfast; BL, before lunch; BD, before dinner; BT, bed time. (b) HbA1c levels. (c) Weight changes. (d) Dose of liraglutide. (e) Dose of basal insulin. (f) Dose of metformin. The circles and squares indicate good-responders and poor-responders, respectively. The opened or closed symbols indicate before or after liraglutide administration, respectively. Adm, admission; Dis, discharge; -3M, -3 months; 0M, 0 month; 3M, 3 months; 6M, 6 months. The bar indicates the S.D. *$p<0.05$ vs. 0M (Dis) in good-responders and poor-responders; †$p<0.05$ for good- vs. poor-responders at each time-point using repeated-measures analysis of variance.

Figure 3. Results of 75-g OGTTs before (1st-) and 2 days after (2nd-) starting liraglutide on good-responders (GR, n=28) and poor-responders (PR, n=13).

Panels (a)-(d) show the comparison of the concentrations of plasma glucose, serum insulin, serum C-peptide and plasma glucagon between before (opened symbols, 1st-OGTT) and 2 days after starting liraglutide (closed symbols, 2nd-OGTT) in good-responders (circle symbols, left panels) and poor-responders (square symbols, right panels), respectively. Panels (e)-(h) show the comparison of the values of the changes from baseline (0 min) to 120 min during the OGTTs in the levels of glucose, insulin, C-peptide and glucagon, indicated as $\Delta Glucose_{120min}$, $\Delta Insulin_{120min}$, $\Delta C\text{-peptide}_{120min}$ and $\Delta Glucagon_{120min}$, between before (white bars, 1st-OGTT) and 2 days after starting liraglutide (black bars, 2nd-OGTT). Panels (i) and (j) show the comparisons of the molar ratio of insulin to glucagon and that of proinsulin to insulin in the fasting sera between before (white bars, 1st-OGTT) and 2 days after starting liraglutide (black bars, 2nd-OGTT). GR, good-responders; PR, poor-responders. The bar indicates the S.D. *$p<0.05$ for 1st- vs. 2nd-OGTT in each group by paired t-test. †$p<0.05$ for good- vs. poor-responders at each time-point by t-test. Panel (k) show the receiver operating characteristic (ROC) curves of "the subtraction of $\Delta Insulin_{120min}$ during 1st-OGTT from $\Delta Insulin_{120min}$ during 2nd-OGTT", i.e. "Increase in

ΔInsulin$_{120min}$", for the good-responder at 6 months after switching the therapy. AUC, area under the curve; CI, confidence interval; PPV, positive-predictive value; NPV, negative-predictive value.

APPENDIX

Supplementary Figure 1.

Modified results of Figure 3(a)-(d), that is, the comparison between good-responders and poor-responders in 75-g OGTTs before (1st-) and 2 days after (2nd-) starting liraglutide.

Panels (a)-(d) show the comparison of the concentrations of plasma glucose, serum insulin, serum C-peptide and plasma glucagon between good-responders (circle symbols) and poor-responders (square symbols) in 1st-OGTT (opened symbols, left panels) and 2nd-OGTT (closed symbols, right panels). The bar indicates the S.D. †$p<0.05$ for good- vs. poor-responders at each time-point by t-test.

Supplementary Figure 2.

ROC analysis of the baseline C-peptide index at fasting for a good-responder, which did not reach a significant level.

Table 1. Baseline features of participants

	Total	**Good responders**	**Poor responders**	**P-value**
n	41	28	13	
Gender (Male:Female)	21:20	15:13	6:7	0.92
Age (years)	62.9 ± 11.0	64.4 ± 8.1	59.8 ± 15.1	0.39
Duration of diabetes (years)	21.2 ± 8.8	20.5 ± 8.0	22.9 ± 10.2	0.48
Duration of insulin therapy (years)	11.3 ± 7.3	10.6 ± 7.3	12.9 ± 7.1	0.26
Duration of BBT (years)	8.6 ± 4.6	8.0 ± 4.0	9.7 ± 5.6	0.46
Height (cm)	160.8 ± 8.1	161.1 ± 6.8	160.3 ± 10.4	0.81
Weight (kg)	76.3 ± 19.3	73.9 ± 13.8	81.3 ± 26.9	0.41
BMI (kg/m^2)	29.4 ± 6.7	28.5 ± 5.3	31.3 ± 8.7	0.27
HbA1c (NGSP, %)	7.6 ± 1.1	7.6 ± 1.2	7.8 ± 1.1	0.54
eGFR (mL/min/1.73m^2)	61.4 ± 18.6	61.1 ± 17.9	62.0 ± 19.8	0.94
C-peptide index at fasting	0.96 ± 0.55	1.01 ± 0.51	0.86 ± 0.60	0.10
C-peptide index at 2h during OGTT	1.68 ± 1.22	1.69 ± 1.02	1.66 ± 1.66	0.46
Proteinuria (g/gCr)	0.58 ± 1.22	0.46 ± 1.11	0.83 ± 1.39	0.33
Retinopathy (n, %)	25 (61.0)	16 (57.1)	9 (69.2)	0.51
Neuropathy (n, %)	27 (65.9)	17 (60.7)	10 (76.9)	0.48
Cardiovascular disease (n, %)	15 (36.6)	9 (32.1)	6 (46.2)	0.49
Pharmacological treatment				
Total insulin (units/day)	47.5 ± 20.8	43.3 ± 17.7	56.8 ± 23.8	0.055
Basal insulin (units/day)	18.9 ± 8.7	17.1 ± 8.4	22.9 ± 7.8	0.028
Bolus insulin (units/day)	28.7 ± 15.6	26.2 ± 12.6	33.9 ± 19.6	0.18
Metformin (n, %)	22 (53.7)	14 (50.0)	8 (61.5)	0.52
Sulfonylurea (n, %)	0 (0.0)	0 (0.0)	0 (0.0)	----
Glinide (n, %)	1 (2.4)	1 (3.6)	0 (0.0)	1.00
DPP4 inhibitor (n, %)	12 (29.3)	6 (21.4)	6 (46.1)	0.15
Pioglitazone (n, %)	5 (12.2)	3 (10.7)	2 (15.4)	0.64
α-GI (n, %)	3 (7.3)	2 (7.1)	1 (7.7)	1.00
SGLT2 inhibitor (n, %)	2 (4.9)	1 (3.6)	1 (7.7)	0.54

BBT, basal-bolus insulin therapy; BMI, body mass index; eGFR, estimated glomerular filtration rate; C-peptide index, 100 × C-peptide (ng/mL)/glucose (mg/dL); OGTT, 75-g oral glucose tolerance test; DPP4, dipeptidyl peptidase-4; α-GI, α-glucosidase inhibitor; SGLT2, sodium-glucose cotransporter 2. The values are given as the means±standard deviations. P-values for differences between good- and poor-responders were calculated using the t-test or Chi-square test.

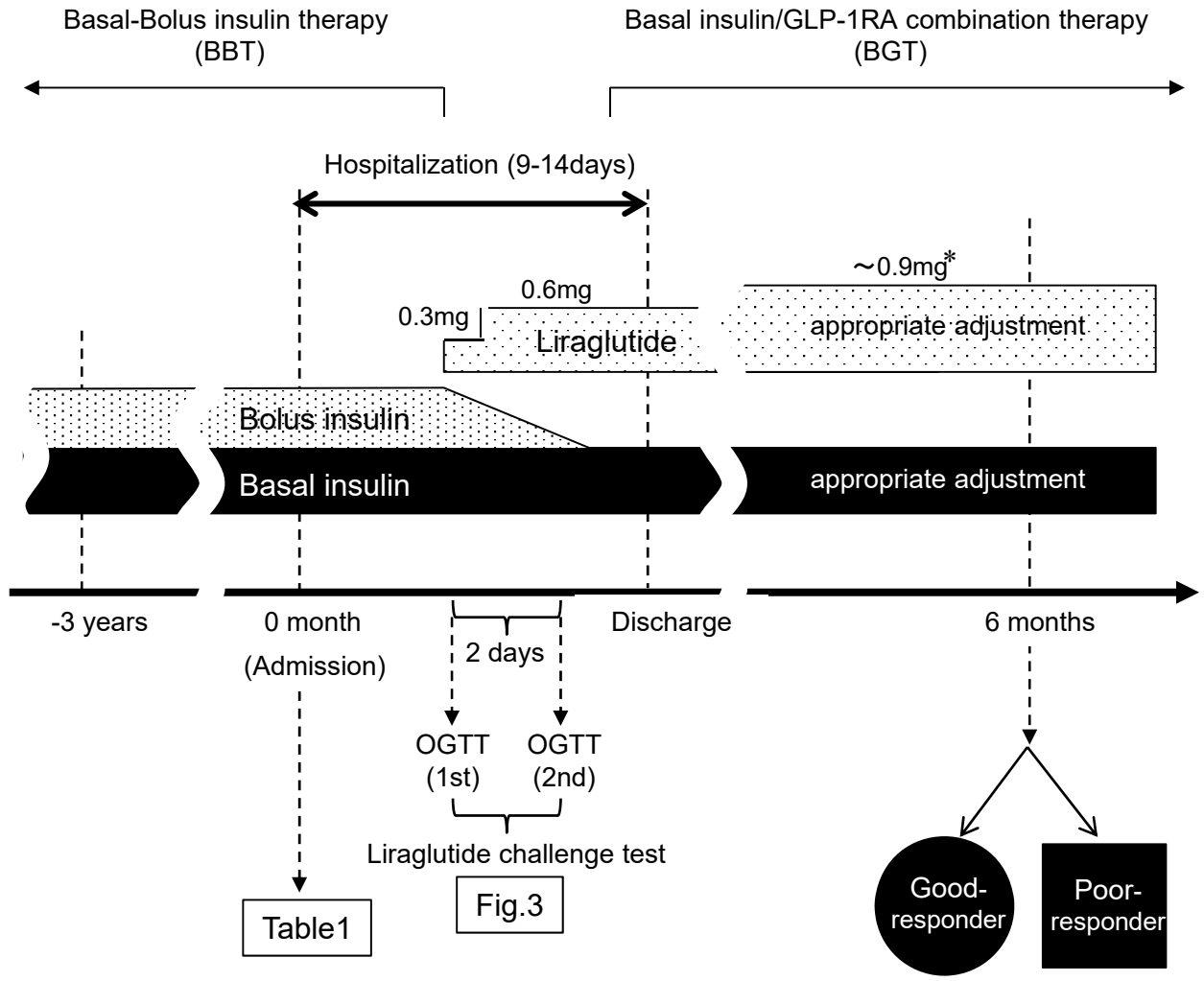
Table 2. Logistic regression analyses of clinical characteristics or insulin/glucagon responses derived from OGTTs to predict good-responders at 6 months after switching from basal-bolus insulin to basal insulin/liraglutide combination therapy.

Predictors of good-responder	Univariate		Multivariate	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age (per 1 year)	1.04 (0.98-1.10)	0.21	1.00 (0.90-1.10)	0.95
Sex (Male)	1.35 (0.36-5.04)	0.66	4.52 (0.58-35.37)	0.13
BMI (per 1 kg/m^2)	0.94 (0.85-1.04)	0.21	1.06 (0.87-1.28)	0.55
Total insulin dose (per 1 unit/day)	0.97 (0.93-1.01)	0.053	0.97 (0.91-1.03)	0.32
Duration of BBT (per 1 year)	0.93 (0.80-1.07)	0.29	0.89 (0.72-1.09)	0.22
Increase in $\Delta Insulin_{120min}$ (per 1 μU/mL)	1.07 (1.01-1.14)	0.003	1.09 (1.01-1.17)	0.018
Decrease in $\Delta Glucagon_{120min}$ (per -1 pg/mL)	1.01 (0.97-1.05)	0.61	1.01 (0.97-1.06)	0.76

BMI, body mass index; BBT, basal-bolus insulin therapy; OR, odds ratio; CI, confidence interval. "Total insulin dose" indicates the total daily dose of insulin needed just before switching therapy. $\Delta Insulin_{120min}$ and $\Delta Glucagon_{120min}$ mean the changes in the levels of insulin and glucagon from baseline (0 min) to 120 min during OGTT, respectively. "Increase in $\Delta Insulin_{120min}$" and "Decrease in $\Delta Glucagon_{120min}$" were calculated as follows. "Increase in $\Delta Insulin_{120min}$" = ($\Delta Insulin_{120min}$ during 2^{nd}-OGTT) – ($\Delta Insulin_{120min}$ during 1^{st}-OGTT); "Decrease in $\Delta Glucagon_{120min}$" = ($\Delta Glucagon_{120min}$ during 2^{nd}-OGTT) – ($\Delta Glucagon_{120min}$ during 1^{st}-OGTT).

Figure 1

(a)


Basal-Bolus insulin therapy
(BBT)
Basal insulin/GLP-1RA combination therapy
(BGT)
Hospitalization (9-14days)
0.6mg
0.3mg
Liraglutide
~0.9mg*
appropriate adjustment
Bolus insulin
Basal insulin
appropriate adjustment
-3 years
0 month
(Admission)
2 days
Discharge
6 months
OGTT
(1st)
OGTT
(2nd)
Liraglutide challenge test
Fig.3
Table1
Good-
responder
Poor-
responder

(b)

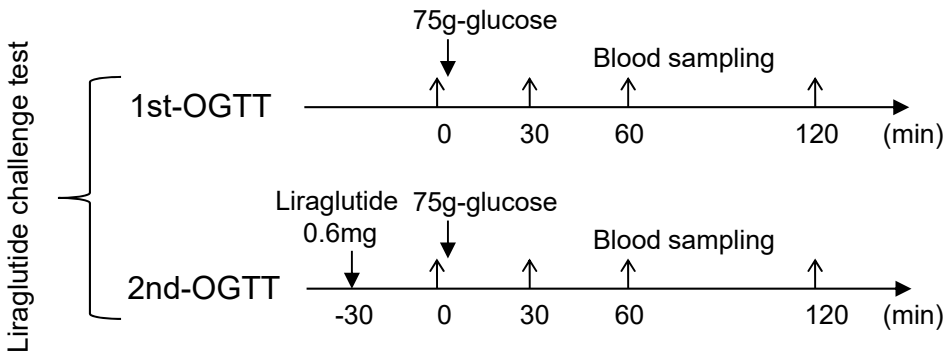

Liraglutide challenge test
1st-OGTT
75g-glucose
Blood sampling
0
30
60
120
(min)
Liraglutide
0.6mg
2nd-OGTT
75g-glucose
Blood sampling
-30
0
30
60
120
(min)

Figure 2

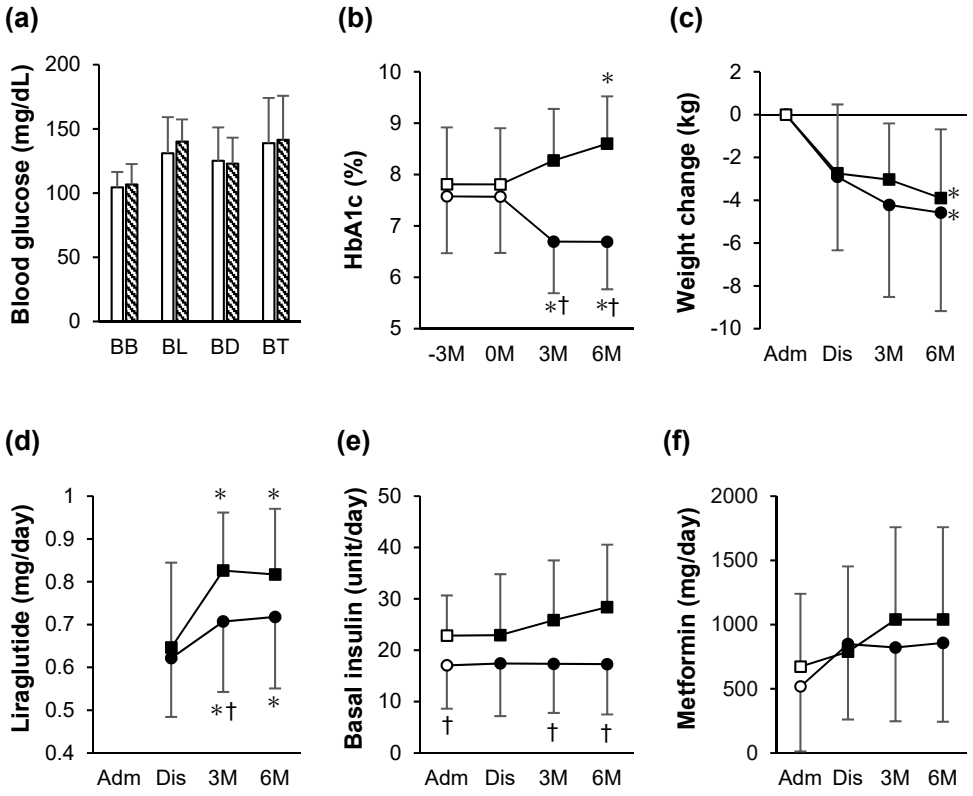
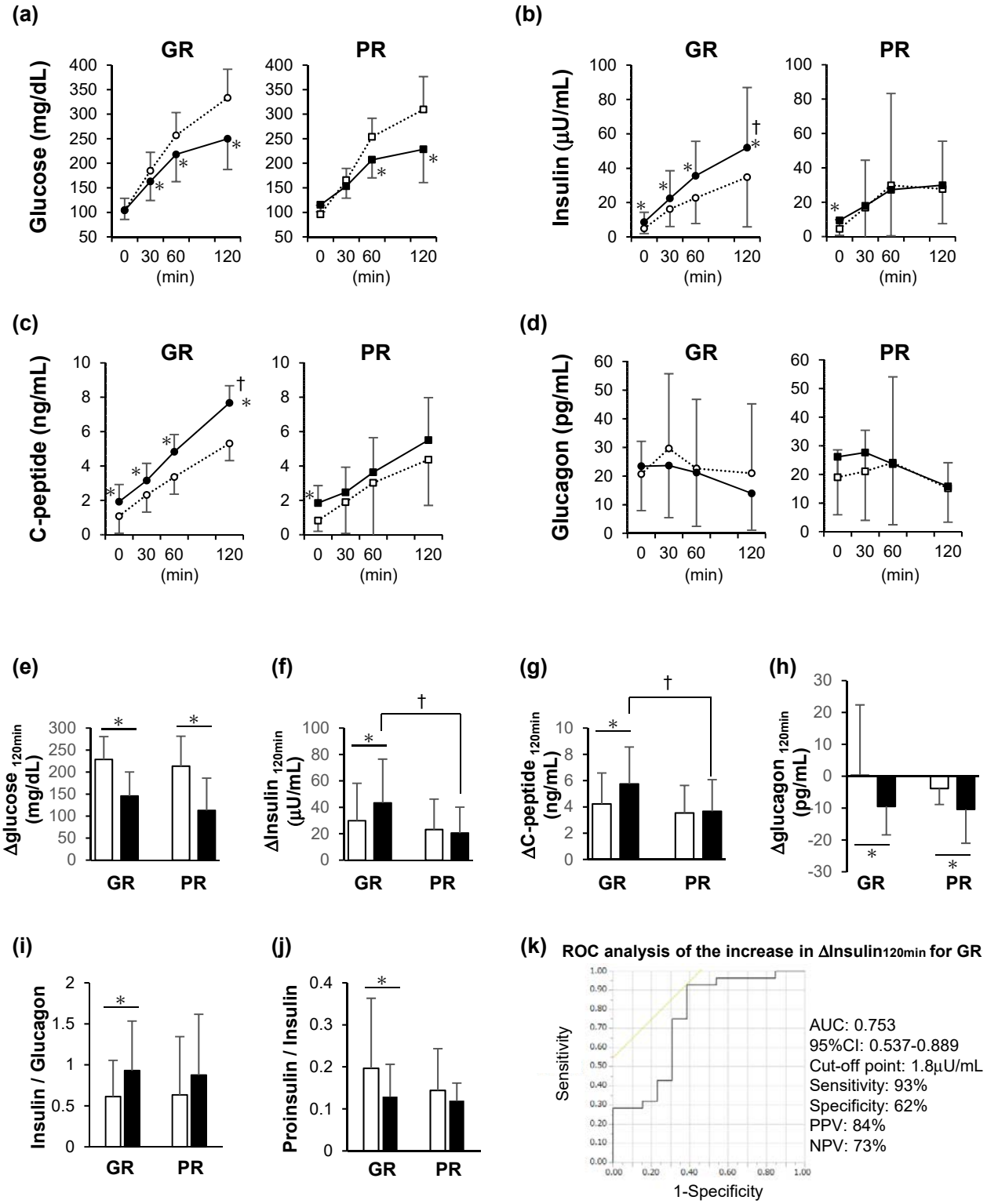
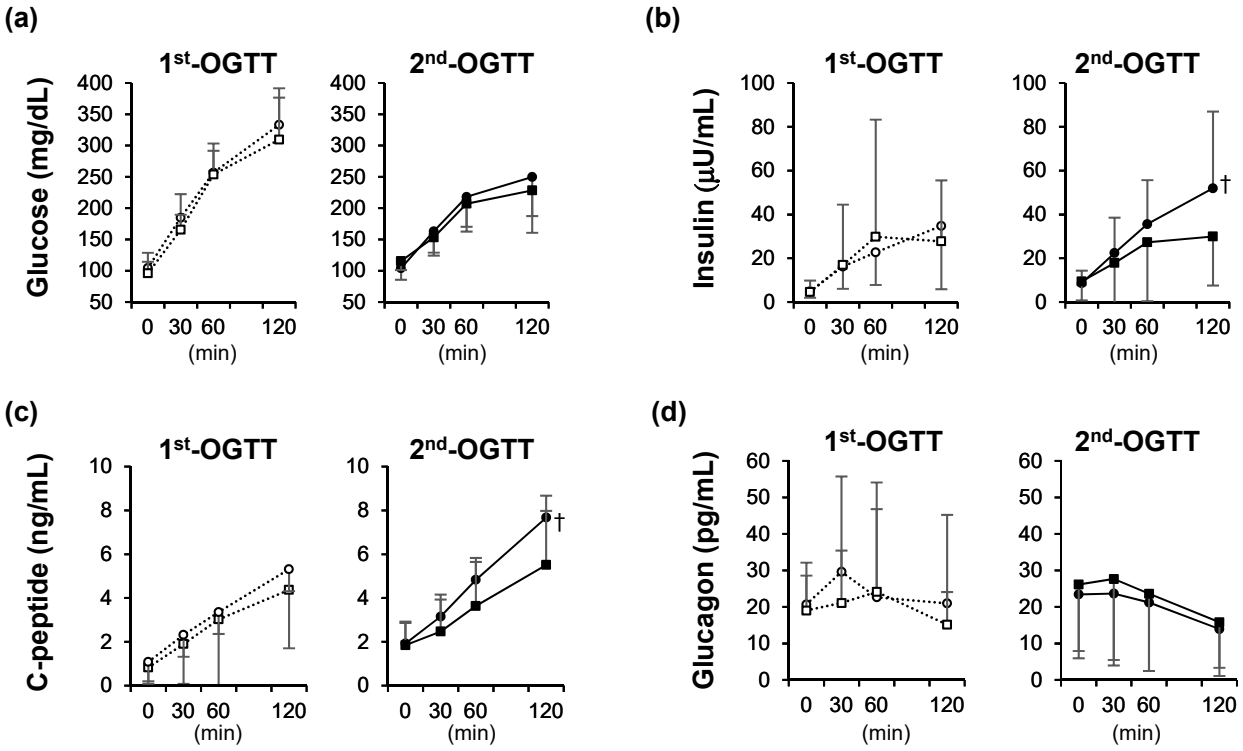

(a)
Blood glucose (mg/dL)
200
150
100
50
0
BB
BL
BD
BT
(b)
HbA1c (%)
10
9
8
7
6
5
*
*†
*†
-3M
0M
3M
6M
(c)
Weight change (kg)
2
0
-2
-4
-6
-8
-10
*
*
Adm
Dis
3M
6M
(d)
Liraglutide (mg/day)
1
0.9
0.8
0.7
0.6
0.5
0.4
*
*
*†
*
Adm
Dis
3M
6M
(e)
Basal insulin (unit/day)
50
40
30
20
10
0
†
†
†
Adm
Dis
3M
6M
(f)
Metformin (mg/day)
2000
1500
1000
500
0
Adm
Dis
3M
6M

Figure 3

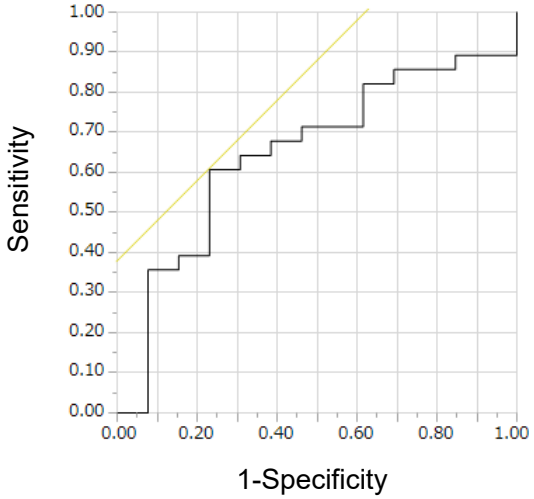

(a)
GR
PR
Glucose (mg/dL)
(min)
(b)
Insulin (μU/mL)
(c)
C-peptide (ng/mL)
(d)
Glucagon (pg/mL)
(e)
Δglucose 120min (mg/dL)
(f)
ΔInsulin 120min (μU/mL)
(g)
ΔC-peptide 120min (ng/mL)
(h)
Δglucagon 120min (pg/mL)
(i)
Insulin / Glucagon
(j)
Proinsulin / Insulin
(k)
ROC analysis of the increase in ΔInsulin120min for GR
Sensitivity
1-Specificity
AUC: 0.753
95%CI: 0.537-0.889
Cut-off point: 1.8μU/mL
Sensitivity: 93%
Specificity: 62%
PPV: 84%
NPV: 73%

Supplementary Figure 1



Supplementary Figure 2

ROC analysis of the baseline C-peptide index for a good-responder



AUC: 0.648
95%CI: 0.451-0.805
Cut-off point: 0.843
Sensitivity: 61%
Specificity: 77%
PPV: 85%
NPV: 48%