# Postprandial serum C-peptide value is the optimal index to identify patients with non-obese type 2 diabetes who require multiple daily insulin injection: Analysis of C-peptide values before and after short-term intensive insulin therapy

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## ABSTRACT

**Aims/Introduction:** Type 2 diabetes is a progressive disease characterized by a yearly decline in insulin secretion; however, no definitive evidence exists showing the relationship between decreased insulin secretion and the need for insulin treatment. To determine the optimal insulin secretory index for identifying patients with non-obese type 2 diabetes who require multiple daily insulin injection (MDI), we evaluated various serum C-peptide immunoreactivity (CPR) values.

**Materials and Methods:** We near-normalized blood glucose with intensive insulin therapy (IIT) over a 2-week period in 291 patients with non-obese type 2 diabetes, based on our treatment protocol. After improving hyperglycemia, we challenged with oral hypoglycemic agent (OHA), and according to the responsiveness to OHA, patients were classified into three therapy groups: OHA alone ($n$ = 103), basal insulin plus OHA (basal insulin-supported oral therapy [BOT]; $n$ = 56) and MDI ($n$ = 132). Glucagon-loading CPR increment (ΔCPR), fasting CPR (FCPR), CPR 2 h after breakfast (CPR2h), the ratio of FCPR to FPG (CPI), CPI 2 h after breakfast (CPI2h) and secretory unit of islets in transplantation (SUIT) were submitted for the analyses. Receiver operating characteristic (ROC) and multiple logistic analyses for these CPR indices were carried out.

**Results:** Many CPR values were significantly lower in the MDI group compared with the OHA alone or BOT groups. ROC and multiple logistic analyses disclosed that post-prandial CPR indices (CPR2h and CPI2h) were the most reliable CPR markers to identify patients requiring MDI.

**Conclusions:** Postprandial CPR level after breakfast is the most useful index for identifying patients with non-obese type 2 diabetes who require MDI therapy. (J Diabetes Invest, doi: 10.1111/jdi.12103, 2013)

**KEY WORDS: C-peptide, Meal load, Multiple daily insulin injection**

## INTRODUCTION

Type 2 diabetes mellitus is a progressive disease characterized by a yearly decline in insulin secretion[1–3]. Parients with type 2 diabetes will eventually require insulin therapy. This insulin therapy can involve various regimens including basal insulin-supported oral therapy (BOT) or multiple daily insulin injection (MDI). The American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) have published a consensus statement[4] regarding the management of hyperglycemia in type 2 diabetes. This includes a practical algorithm of the therapy, based on blood glucose and glycated hemoglobin ($HbA_{1c}$), which progresses from oral hypoglycemic agent (OHA) to basal insulin therapy, and then to MDI. The progression from OHA to MDI in type 2 diabetes is assumed to be closely related to the decrease in insulin secretory capacity. In clinical practice, whether or not insulin therapy is required for glycemic control is a significant issue for patients and physicians, yet no useful insulin secretory index for identifying when insulin therapy should be started exists. Recently, regarding serum C-peptide immunoreactivity (CPR) as a marker for predicting insulin requirement in type 2 diabetes, several

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reports have been published[5–7], where useful CPR indices were advocated. In the present study, to determine the optimal CPR index for identifying MDI-requiring patients with non-obese type 2 diabetes, we retrospectively analyzed various serum CPR values by comparing the values among different diabetes therapy groups, which were determined according to our treatment protocol. The protocol consists of intensive insulin therapy (IIT) and challenge of OHA mainly including insulin secretagogues after IIT.

## MATERIAL AND METHODS

### Patients

Using our department diabetes database, we initially selected 1,039 patients with type 2 diabetes who had been hospitalized and treated with insulin for poor glycemic control over a 36-month period between October 2007 and September 2010. Among this group, those with incomplete plasma glucose (PG) values (163 patients) or CPR (89 patients), or those in a preoperative state (109 patients) were excluded, leaving 678 patients. Then, another 109 patients with conditions influencing CPR assessment or selection of insulin therapy, including those with chronic liver disease (37), malignancies (32), dementia (13), acute infections (11), diabetic foot (8) or who deviated from the treatment protocol (8), were also excluded from the study, leaving 569 patients. Of these, 291 non-obese (body mass index [BMI] of <25) patients with type 2 diabetes were enrolled in the study.

The mode of treatment at baseline in these cases was OHA alone in 160 patients (a sulfonylurea in 115), insulin in 62 patients (combined with OHA in 21) and no medical treatment in 69 patients. Table 1 shows the baseline clinical characteristics and type of treatment at enrolment in these patients.

**Table 1** | Baseline clinical characteristics of patients enrolled in the study ($n$ =291)

| | |
|---|---|
| Male/female | 181/110 |
| Age (years) | 63.1 ± 11.1 |
| Period from diagnosis of diabetes (years) | 12.4 ± 10.1 |
| BMI (kg/m$^2$) | 21.7 ± 2.2 |
| FPG (mg/dL) | 184 ± 59 |
| $HbA_{1c}$ (%, NGSP) | 10.3 ± 1.9 |
| FCPR (ng/mL) | 1.32 ± 0.86 |
| ΔCPR (ng/mL) | 1.83 ± 1.18 |
| CPR2h (ng/mL) | 3.70 ± 2.11 |
| CPI (ng/mg) | 0.78 ± 0.57 |
| CPI2h (ng/mL) | 1.40 ± 1.03 |
| SUIT (%) | 20.1 ± 18.3 |
| Diabetes treatment, $n$ (%) | |
| Diet only | 69 (23.7) |
| OHA alone | 160 (54.9) |
| Sulfonylurea ($n$) | (115) |
| Insulin | 62 (21.3) |
| With OHA ($n$) | (21) |
| Complicated diseases, $n$ (%) | |
| Peripheral neuropathy | 154 (56.4) |
| Diabetic retinopathy | 137 (47.4) |
| Diabetic renal disease | 84 (28.9) |
| Ischemic heart disease | 47 (16.7) |
| Cerebral vascular disease | 24 (8.5) |
| Arteriosclerotic disease of the legs | 23 (8.3) |

Data are shown as mean ± standard deviation; number of patients and percentages in parenthesis. ΔCPR, increment of C-peptide immunoreactivity during glucagon test; BMI, body mass index; CPI, C-peptide index; CPI2h, C-peptide index 2 h after breakfast; CPR2h, C-peptide immunoreactivity 2 h after breakfast; FCPR, fasting C-peptide; FPG, fasting plasma glucose; $HbA_{1c}$, glycated hemoglobin; NGSP, National Glycohemoglobin Standardization Program; OHA, oral hypoglycemic agent; SUIT, secretory unit of islets in transplantation.

### Treatment Protocol

Treatment proceeded based on a 2-week treatment protocol. On day 1, a standard diabetes meal, 30 kcal/kg of standard bodyweight: 22 × body height (m)$^2$, consisting of 62% carbohydrate, 16% protein and 22% fat (when taking 1600 kcal diet per day), was started. Patient self-measurement of capillary blood glucose four times daily (before each meal and at bedtime) by using a portable blood glucose monitor (Glutest Ace R; Sanwa Kagaku Co. Ltd., Nagoya, Japan) was also started. On day 2, PG excursion at 7 points (before 3 meals, 2 h after starting meals, and at 22.00 hours) was measured under the baseline treatment of OHAs or insulin injections. On the morning of day 3, previous treatments were discontinued and a glucagon-loading test was carried out. Thereafter, IIT was started as described here.

In all patients, injections before each meal with 4 U of regular or ultra short-acting analog insulin, and at 22.00 hours with 4 U of intermediate-acting insulin (NPH), insulin glargine or insulin detemir were started. The insulin dose was adjusted daily for a target glucose value of 110 mg/dL before the three meals.

On days 8–9, insulin was discontinued and OHA was started, consisting of either 40–80 mg gliclazide, 1–2 mg glimepiride, 270 mg nateglinide or 30 mg mitiglinide in combination with either 150–300 mg acarbose, 0.9 mg voglibose or 150 mg miglitol (daily doses of each). These medicines were administered for 3 days, and if glucose values were equal to the values obtained with IIT, the OHA treatment was continued. If glucose was ≥140 mg/dL before breakfast, the original bedtime insulin (same type and dose at 22.00 hours) was added. If glucose was ≥140 mg/dL both before breakfast and before dinner, all OHAs were discontinued, and a biphasic analog-mixed insulin, at 80% of the total daily insulin dose before switching, split in a 2:1 ratio, was started before breakfast and before dinner. When, despite two injections of the insulin, appropriate blood glucose levels were not achieved, either half of the morning dose of the same insulin was added as a third injection before lunch, or the four times daily insulin regimen used before switching was resumed. On day 13, PG excursion was measured again, and final adjustments to OHAs or insulin dose were made. According to the

protocol, the final treatment regimen was divided into three groups: OHA alone, basal insulin plus OHA (BOT) and insulin two to four times daily (MDI).

Patients previously admitted to our hospital, and who were at that time assessed as requiring MDI, or in whom two physicians, including a diabetologist certified by the Japan Diabetes Association, judged MDI necessary were continued on insulin therapy without OHA challenge.

### PG and CPR Sampling

On day 2, PG (mg/mL) excursion was measured at 7 points. At that time, fasting CPR (FCPR; ng/mL) before breakfast and CPR 2 h after starting the meal (CPR2h) were measured. On day 3, under fasting conditions, an intravenous glucagon (1 mg) loading test was carried out, and CPR was measured at 0 and 6 min. On day 13, PG excursion, FCPR and CPR2h were also measured.

The CPR indices submitted for analysis included the glucagon loading CPR increment (ΔCPR) on day 3; and FCPR, CPR2h, the ratio of FCPR to fasting plasma glucose (FPG): C-peptide index (CPI, FCPR/FPG × 100, ng/mg)[7], CPI2h (CPI 2 h after breakfast) and the secretory units of islets in transplantation (SUIT, %)[8], which was calculated by the formula: 1485 × FCPR/(FPG − 61.8)[8], on day 2 and day 13.

C-peptide immunoreactivity was measured by radioimmunoassay (RIA) using a C-Peptide Kit 'Daiichi' III (TFB Inc., Tokyo, Japan). Seven points of daily PG excursion values before starting and after completing IIT were determined in venous blood by the hexokinase method.

### Statistical Analysis

The clinical characteristics of participants used in the analysis were age, period from diagnosis of diabetes (disease period), BMI, FPG, $HbA_{1c}$ (National Glycohemoglobin Standardization Program [NGSP] value), serum CPR concentrations and calculated CPR values. Among the OHA alone, BOT and MDI groups, clinical markers and individual CPR values were analyzed using ANOVA. Also, intergroup differences between the OHA group (defined as the OHA alone group combined with the BOT group) and MDI group were analyzed using a non-paired *t*-test. In addition, to uncover indices capable of discriminating the requirement for MDI treatment from that of other treatments, receiver operating characteristic (ROC) and multiple logistic regression analyses of each CPR index were carried out. The contribution of disease period to necessity of MDI was tested with ROC analysis as well. The statistical software used for analyses were Excel Statistics 2010 for Windows version 1.09 (Social Survey Research Information Co. Ltd., Tokyo, Japan) and IBM SPSS Statistics version 19 (IBM Japan, Tokyo, Japan). $HbA_{1c}$ values were converted from Japan Diabetes Society to NGSP values by the conversion equation[9].

The clinical study and treatment protocol were submitted to the Clinical Research Approval Committee, and approved by the Medical Ethics Committee of Kurashiki Central Hospital. Before initiation of treatment, the attending physician provided a written explanation of the study protocol and verbal consent was obtained from all patients.

## RESULTS

### Daily Plasma Glucose Excursions Before and After IIT

Under the baseline treatment conditions before starting IIT, the daily PG excursion values at the 7 points were 184 ± 59, 293 ± 95, 253 ± 94, 264 ± 109, 200 ± 84, 266 ± 89 and 252 ± 85 mg/dL (mean ± standard deviation [SD]) in all participants. Under the final treatment conditions assigned after IIT, values were 117 ± 22, 174 ± 52, 142 ± 42, 175 ± 54, 135 ± 44, 169 ± 54, and 162 ± 51 mg/dL (mean ± SD). Significant decreases were observed at all points ($P < 0.01$).

### Clinical Characteristics and CPR Levels According to Treatment Groups

Of the 291 patients, the number in each final therapy was: OHA alone, 103; BOT, 56; and MDI, 132 (2 insulin injections per day, 95; 3 injections, 15; and 4 injections, 22). The relationships between baseline treatment and each final therapy are shown in Figure S1.

Details of oral agents used in the OHA alone group are shown in Table S1a, and details of oral agents combined with

**Table 2** | Baseline clinical profiles and C-peptide immunoreactivity levels in each final therapy group

| Indices | OHA alone ($n$ = 103) | BOT ($n$ = 56) | MDI ($n$ = 132) |
|---|---|---|---|
| Male/female | 67/36 | 37/19 | 77/55 |
| Age (years) | 63.5 ± 9.9 | 57.4 ± 12.8** | 65.2 ± 10.4†† |
| Period from diagnosis (years) | 8.0 ± 8.7 | 10.4 ± 8.0 | 16.5 ± 10.2**†† |
| BMI (kg/m$^2$) | 21.8 ± 2.0 | 22.8 ± 1.8* | 21.2 ± 2.4†† |
| FPG (mg/dL) | 162 ± 39 | 204 ± 56** | 193 ± 67** |
| $HbA_{1c}$ (%; NGSP) | 10.0 ± 1.8 | 10.5 ± 1.9 | 10.3 ± 2.1 |
| FCPR (ng/mL) | 1.41 ± 0.61 | 1.55 ± 1.12 | 1.15 ± 0.86† |
| ΔCPR (ng/mL) | 2.28 ± 1.22 | 2.19 ± 1.18 | 1.33 ± 0.92**†† |
| CPR2h (ng/mL) | 4.43 ± 1.69 | 4.35 ± 2.16 | 2.86 ± 2.09**†† |
| CPI (ng/mg) | 0.91 ± 0.43 | 0.81 ± 0.63 | 0.66 ± 0.61** |
| CPI2h (ng/mg) | 1.87 ± 1.03 | 1.55 ± 0.93 | 0.97 ± 0.89**†† |
| SUIT (%) | 23.8 ± 13.2 | 19.5 ± 19.2 | 17.5 ± 20.8* |

Baseline clinical makers and C-peptide immunoreactivity (CPR) levels in each final therapy group are shown. Data are presented as mean ± standard deviation. Statistical analyses were carried out by ANOVA and post-hoc comparison by the Bonferroni method. *$P < 0.05$, **$P < 0.01$ vs oral hypoglycemic agent (OHA) alone; †$P < 0.05$, ††$P < 0.01$ vs basal insulin-supported oral therapy (BOT). ΔCPR, increment of C-peptide immunoreactivity during glucagon test; BMI, body mass index; CPI, C-peptide index; CPI2h, C-peptide index 2 h after breakfast; CPR2h, C-peptide immunoreactivity 2 h after breakfast; FCPR, fasting C-peptide; FPG, fasting plasma glucose; $HbA_{1c}$, glycated hemoglobin; MDI, multiple daily insulin injection; SUIT, secretory unit of islets in transplantation.

**Table 3** | Comparison of clinical markers and C-peptide immunoreactivity levels, before and after intensive insulin therapy, between the oral hypoglycemic agent (oral hypoglycemic agent alone + basal insulin-supported oral therapy) and multiple daily insulin injection groups

| Indices | OHA alone + BOT ($n$ = 159) | MDI ($n$ = 132) | Non-paired $t$-test $P$-value |
|---|---|---|---|
| Period from diagnosis (years) | 8.8 ± 8.5 | 16.5 ± 10.2 | <0.01 |
| BMI (kg/m$^2$) | 22.2 ± 2.0 | 21.2 ± 2.4 | <0.01 |
| CPR levels before IIT | | | |
| FCPR (ng/mL) | 1.46 ± 0.83 | 1.15 ± 0.86 | <0.01 |
| ΔCPR (ng/mL) | 2.25 ± 1.20 | 1.33 ± 0.92 | <0.01 |
| CPR2h (ng/mL) | 4.40 ± 1.86 | 2.86 ± 2.09 | <0.01 |
| CPI (ng/mg) | 0.87 ± 0.51 | 0.66 ± 0.61 | <0.01 |
| CPI2h (ng/mL) | 1.76 ± 1.00 | 0.97 ± 0.89 | <0.01 |
| SUIT (%) | 22.3 ± 15.6 | 17.5 ± 20.8 | <0.05 |
| CPR levels after IIT | | | |
| FCPR | 1.33 ± 0.78 | 0.70 ± 0.48 | <0.01 |
| CPR2h (ng/mL) | 5.10 ± 2.16 | 2.10 ± 1.39 | <0.01 |
| CPI (ng/mg) | 1.13 ± 0.66 | 0.59 ± 0.39 | <0.01 |
| CPI2h (ng/mL) | 3.17 ± 1.46 | 1.20 ± 0.79 | <0.01 |
| SUIT (%) | 37.8 ± 23.3 | 19.7 ± 13.0 | <0.01 |

C-peptide immunoreactivity (CPR) index levels, before and after intensive insulin therapy, and other markers in the oral hypoglycemic agent (OHA) alone + basal insulin-supported oral therapy (BOT; OHA group) and multiple daily insulin injection (MDI) group are shown as mean ± standard deviation, respectively. ΔCPR, increment of C-peptide immunoreactivity during glucagon test; BMI, body mass index; CPI, C-peptide index; CPI2h, C-peptide index 2 h after breakfast; CPR2h, CPR 2 h after breakfast; FCPR, fasting C-peptide; SUIT, secretory unit of islets in transplantation.

insulin and basal insulin remedies in the BOT group are shown in Table S1b. Details of insulin therapy modes in the MDI group are shown in Table S1c.

Table 2 shows the baseline clinical characteristics in the three groups, including the following six indices: FCPR, ΔCPR, CPR2h, CPI, CPI2h and SUIT. ANOVA analysis among the three groups showed that, in the MDI group, the period from diagnosis was longer and BMI was smaller compared with the OHA alone and/or BOT group ($P < 0.01$), whereas $HbA_{1c}$ levels were not different among the groups. Analysis of CPR values showed no significant differences between the OHA alone and BOT groups, whereas all CPR values were significantly lower in the MDI group ($P < 0.01$; $P < 0.05$, in SUIT) than in the OHA alone group, except for FCPR. CPI and SUIT did not significantly differ in the BOT and MDI groups, whereas all other values were lower in the MDI group ($P < 0.01$; $P < 0.05$, in FCPR).

Because no differences were observed in any of the CPR indices between the OHA alone and BOT groups, and because both groups were responsive to OHAs, they were combined to form the OHA group. As shown in Table 3, a comparison of the clinical characteristics and CPR values between the OHA and MDI groups showed that the period from diagnosis was longer, BMI was smaller, and all CPR values before IIT ($P < 0.01$; $P < 0.05$, in SUIT) and after IIT ($P < 0.01$) were lower in the MDI group compared with the OHA group.

### ROC Analysis of CPR Indices to Determine the Requirement for MDI Before and After IIT

In ROC analysis of CPR indices using the baseline data before IIT, as shown in Table 4, the area under the curve (AUC) and specificity were: ΔCPR, 0.742 and 69.2% (cut-off, 1.5 ng/mL); CPR2h, 0.752 and 82.4% (3.0 ng/mL); CPI, 0.692 and 68.6% (0.6 ng/mg); CPI2h, 0.779 and 79.2% (1.0 ng/mg); SUIT, 0.677 and 62.3% (15%), respectively. AUC and specificity were higher in both CPR2h and CPI2h compared with the other three indices. Sensitivity for all indices was approximately 60%. In ROC analysis of the data after IIT, as shown in Table 5, the AUC and specificity were: CPR2h, 0.902 and 86.8% (cut-off, 3.0 ng/mL); CPI, 0.811 and 81.8% (0.6 ng/mg); CPI2h, 0.912 and 76.1% (2.0 ng/mg); and SUIT, 0.807 and 83.0% (20%), respectively. Although almost all indices showed increases in AUC, sensitivity or specificity compared with baseline, CPR2h and CPI2h were still superior to the others. Figure S2a and S2b show the ROC curves of these indices. Disease period contributed to identifying

**Table 4** | Receiver operating characteristic analysis of disease period and C-peptide immunoreactivity indices for identifying patients who require multiple daily insulin injection therapy, using C-peptide immunoreactivity data before intensive insulin therapy

| Indices | AUC (95% CI) | $P$-value | Cut-off | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|
| Disease period | 0.730 (0.672–0.787) | <0.001 | 15 (years) | 52.8 | 77.0 |
| ΔCPR | 0.742 (0.685–0.800) | <0.001 | 1.5 (ng/mL) | 66.7 | 69.2 |
| CPR2h | 0.752 (0.695–0.809) | <0.001 | 3.0 (ng/mL) | 61.4 | 82.4 |
| CPI | 0.692 (0.630–0.755) | <0.001 | 0.6 (ng/mg) | 65.2 | 68.6 |
| CPI 2 h | 0.779 (0.726–0.832) | <0.001 | 1.0 (ng/mg) | 62.1 | 79.2 |
| SUIT | 0.677 (0.614–0.739) | <0.001 | 15 (%) | 65.2 | 62.3 |

Results of receiver operating characteristic (ROC) analysis using the data before intensive insulin therapy (IIT) are shown (refer to Figure S2a). ΔCPR, increment of C-peptide immunoreactivity during glucagon test; AUC, area under the ROC curve; CPI, C-peptide index; CPI2h, C-peptide index 2 h after breakfast; CPR2h, C-peptide immunoreactivity 2 h after breakfast; SUIT, secretory unit of islets in transplantation.

**Table 5** | Receiver operating analysis of C-peptide immunoreactivity indices for identifying patients who require multiple daily insulin injection therapy, using C-peptide immunoreactivity data after intensive insulin therapy

| Indices | AUC (95% CI) | *P*-value | Cut-off | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|
| CPR2h | 0.902 (0.866–0.939) | <0.001 | 3.0 (ng/mL) | 78.0 | 86.8 |
| CPI | 0.811 (0.761–0.861) | <0.001 | 0.6 (ng/mg) | 65.2 | 81.8 |
| CPI2h | 0.912 (0.879–0.945) | <0.001 | 2.0 (ng/mg) | 87.1 | 76.1 |
| SUIT | 0.807 (0.757–0.857) | <0.001 | 20 (%) | 62.9 | 83.0 |

Results of receiver operating characteristic (ROC) analysis using the data after intensive insulin therapy (IIT) are shown (refer to Figure S2b). ΔCPR, increment of C-peptide immunoreactivity during glucagon test; AUC, area under the ROC curve; CPI, C-peptide index; CPI2h, C-peptide index 2 h after breakfast; CPR2h, C-peptide immunoreactivity 2 h after breakfast; SUIT, secretory unit of islets in transplantation.

**Table 6** | Summary of multiple logistic analyses of C-peptide immunoreactivity indices using (a) before and (b) after intensive insulin therapy data for identifying patients who require multiple daily insulin injection therapy

| Parameters | Partial regression coefficient (95% CI) | Standardized partial regression coefficient | *P*-value | Odds ratio (95% CI) |
|---|---|---|---|---|
| **(a)** | | | | |
| ΔCPR | −0.6932 (−1.0071 ~ −0.3794) | −0.7892 | <0.01 | 0.4999 (0.3653 ~ 0.6843) |
| CPR2h | −0.3786 (−0.5536 ~ −0.2037) | −0.7875 | <0.01 | 0.6848 (0.5749 ~ 0.8157) |
| CPI | −0.6385 (−1.2051 ~ −0.0719) | −0.3520 | <0.05 | 0.5281 (0.2997 ~ 0.9306) |
| CPI2h | −1.0778 (−1.5206 ~ −0.6350) | −1.0864 | <0.01 | 0.3403 (0.2186 ~ 0.5300) |
| SUIT | −0.0155 (−0.0327 ~ −0.0017) | −0.2858 | 0.0776 | 0.9846 (0.9678 ~ 1.0017) |
| **(b)** | | | | |
| CPR2h | −1.0624 (−1.3329 ~ −0.7918) | −2.3746 | <0.01 | 0.3456 (0.2637 ~ 0.4530) |
| CPI | −2.0601 (−2.8288 ~ −1.2914) | −1.2475 | <0.01 | 0.1274 (0.0591 ~ 0.2749) |
| CPI2h | −1.8840 (−2.3879 ~ −1.3801) | −2.8252 | <0.01 | 0.1520 (0.0918 ~ 0.2516) |
| SUIT | −0.6160 (−0.0853 ~ −0.0379) | −1.3216 | <0.01 | 0.9402 (0.9182 ~ 0.9628) |

The summary of multiple-logistically analyzed data of C-peptide immunoreactivity (CPR) parameters cited from Tables S2a (a) and S2b (b) is shown. ΔCPR, increment of C-peptide immunoreactivity during glucagon test; CI, confidence interval; CPI, C-peptide index; CPI2h, C-peptide index 2 h after breakfast; CPR2h, C-peptide immunoreactivity 2 h after breakfast; IIT, intensive insulin therapy; SUIT, secretory unit of islets in transplantation.

patients requiring MDI (AUC 0.730, sensitivity 52.8% and specificity 77.0% at cut-off of 15 years); however, the AUC and specificity were lower compared with that of the main CPR indices (Table 4).

### Multiple Logistic Analysis of CPR Indices to Determine the Requirement for MDI Before and After IIT

Multiple logistic analysis of CPR indices before and after IIT to determine the requirement for MDI therapy was carried out. The summarized results are shown in Table 6. In order to avoid multicollinearities lying between CPR indices, each CPR was separately analyzed together with the clinical markers BMI and disease period in common (refer to Tables S2a and S2b). Of the five CPR parameters before IIT, ΔCPR, CPR2h, CPI and CPI2h were selected as significant explanatory variables; however, a standardized partial regression coefficient was advantageous in ΔCPR, CPR2h and especially CPI2h. Of the four CPR parameters after IIT, all were selected as significant explanatory variables; however, a standardized partial regression coefficient was advantageous in CPR2h and CPI2h.

## DISCUSSION

In this study, the CPR indices that best discriminated the requirement for MDI from the other treatments were CPR2h and CPI2h. Patients of the MDI group had a longer diabetes duration, were lean and characteristically had lower baseline CPR levels, when comparing clinical profiles and CPR levels in three patient groups that were assigned after IIT: OHA alone, BOT or MDI. A limitation of the present study might be that the final treatment regimen was determined in a short space of 3 days. Nevertheless, the present study design in which after near-normalization with IIT under a strict diet, rapidly effective oral agents, such as sulfonylurea (SU) or glinide, were used[4] could help determine which CPR indices contributed to distinguishing the three therapy groups, and to identifying patients who require MDI.

The reasons we targeted type 2 diabetes with a BMI of <25 in our study design are that although obesity has recently been increasing in the Japanese, they have traditionally been non-obese, and that because a large BMI and liver insulin resistance influences daily insulin requirement in type 2 diabetes[10], obesity

might lead to bias in treatment selection. Enrolment of only for non-obese patients, however, would narrow the applicability of our results for clinical use. Therefore, further analysis of the treatment for obese compared with non-obese type 2 diabetes will be required.

CPR indices judged to be the most useful for MDI therapy were postprandial CPR levels; total CPR concentration after a meal consists of postprandial glucose-stimulated insulin secretion and glucose-dependent insulin secretion by incretin[11]. Of these insulin secretion mechanisms, regarding the latter, Bagger *et al.*[12] recently reported that the regulation of incretin effect was impaired in patients with type 2 diabetes. The advantage of indices CPR2h and CPI2h, which were obtained with the physiological meal load unlike the other indices, might have reflected dysfunction of these two mechanisms. Although, in patients with long-term diabetes, such as the MDI group, whether or not the incretin effect further decreases remains to be elucidated[13].

Funakoshi *et al.*[6] carried out ROC analysis of CPR values as indices indicative of insulin therapy in type 2 diabetes, and found CPI to be superior among several CPR markers. They noted that because CPI could be calculated solely from a 1-point blood sample, it was convenient and less burdensome. In their study, however, CPR2h and CPI2h were not provided as CPR indices. Saisho *et al.*[5] reported that postprandial CPR to plasma glucose ratio was the best predictor of subsequent insulin treatment in type 2 diabetes. Although their method that determined requirement for insulin therapy was different from ours, the usefulness of postprandial CPI agreed with our results in the present study. Meier *et al.*[14] analyzed the relationship between CPR indices and human pancreatic β-cell area (determined from surgical specimens); in comparison to fasting measures, such as CPI, CPI 15 or 30 min after oral glucose loading showed better correlation with β-cell area. This shows that the postprandial CPI plays a significant role. Funakoshi *et al.*[15] compared postprandial CPR (PPCPR) to glucagon-loading CPR (CPR6min) in type 2 diabetes, and showed that PPCPR level was influenced by chronic hyperglycemia (estimated with $HbA_{1c}$) to a greater extent than CPR6min level, and was more subject to glucose toxicity than CPR6min or FCPR. These results are interesting for us, considering the improved utility of CPR2h after IIT in the present study. While glucagon loading is a non-physiological test, although the ΔCPR value obtained from this test is one of the confirmed indices estimating a yearly decline of endogenous insulin secretion[16], the utility of the value as an indicator for MDI was inferior to the postprandial indices as shown by the present ROC analysis.

By near-normalization of blood glucose with IIT, a diminution of glucose toxicity and recovery of pancreatic β-cell function can be expected[17,18]. In the MDI group, however, baseline CPR levels were originally low, and even with IIT CPR levels remained low (Table 3), whereas in the OHA group all CPR levels, except for FCPR, were elevated (statistical analysis was not carried out because this point was not within the scope of the work). As shown in Tables 4 and 5, ROC analysis showed that after IIT, compared with before IIT, the AUC of each CPR index increased. This was presumed to contribute to the recovery of CPR levels after ITT in the OHA group, but not in the MDI group.

Similarities in all measured CPR levels were observed in the OHA alone and BOT groups, and both groups were responsive to OHAs. Therefore, the OHA alone and BOT groups were combined to form the OHA group. Even though CPR levels were similar in the two groups, basal insulin injection was required in the BOT group. The reason is because BMI and baseline FPG were significantly higher in the BOT group, as shown in Table 2, and because liver insulin resistance is one of the main pathophysiological features in the obese patients; therefore, basal insulin injection was required to suppress hepatic glucose output (HGO)[19] in the somewhat heavier BOT group. Combination therapy with basal insulin plus oral agents using bedtime NPH insulin and daytime SU originated in North America and Northern Europe[19–21], and the clinical utility of this regimen has been shown[22,23]. Currently, the long-acting analog insulin glargine or detemir is used as basal insulin because of the convenience and efficacy, and the combination therapy with OHA is termed BOT. However, the clinical characteristics and insulin secretory ability of type 2 diabetics responding positively to BOT have not been thoroughly investigated[20]. The present results showed that insulin secretion in patients assigned to BOT was clearly sustained compared with the MDI group, and was similar to the OHA alone group. The clinical marker distinguishing the BOT group from the OHA alone group was not CPR, but rather BMI and FPG.

Incretin-related agents, such as dipeptidyl peptidase-4 (DPP-4) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists, have recently been introduced; however, these agents were not an option in the treatment protocol of the present study. Incretin agents, unlike other antidiabetic agents, exert GLP-1 effects on glucose-dependent insulin secretion and pancreatic β-cell protection[24], and thus might influence selection of patients for insulin therapy in type 2 diabetes. Kozawa *et al.*[25] reported, however, that patients with decreased insulin secretion showed lowered efficacy of GLP-1 receptor agonist, liraglutide. An exploratory study[26] using another GLP-1 receptor agonist, exenatide, showed that insulin-treated type 2 diabetes deteriorated in glucose control in 38% of the patients who switched from insulin to exenatide. These studies suggest that caution is required when switching to injection of a GLP-1 receptor agonist in MDI-requiring patients. Administration of a DPP-4 inhibitor can enhance the action of SU; therefore, in BOT patients taking SU, another treatment option might be possible. In a 24-week study of the effects of 100 mg sitagliptin co-administration on insulin in type 2 diabetic patients[27], FPG and $HbA_{1c}$ were significantly improved; however, the total daily insulin dose did not change, nor did insulin elimination occur.

In the present study, we reported the optimal CPR indices, as well as their cut-off values, for determining the need for insulin therapy. However, according to the results of ROC analysis, as shown in Tables 4 and 5, the sensitivity and specificity of the CPR indices was 60–80% at a cut-off value, which might not always be practical. Therefore, caution is advised in basing the need for insulin therapy in any given patient solely on CPR values. Because of the difficulty in routinely estimating insulin sensitivity at the bedside[28], the evaluation was not included in the present study. However, reports have shown that even with IIT, insulin sensitivity was only partially reversed[17], or was not improved[29]; accordingly, the present data suggests that pancreatic β-cell dysfunction contributes most to the selection of treatment regimen.

Some discrepancies existed between the present results and those of other reports[5–7] in baseline CPR levels and cut-off values of CPR indices for MDI in ROC analysis. Lower FCPR and CPI levels at baseline in the present study were mainly caused by lower average BMI of the participants, and lower CPI cut-off value might be attributable to the methodology in determining insulin requirements, which was different from that of other reports.

In the MDI group, as shown in Table S3, serum creatinine concentration was higher, and creatinine clearance was lower compared with the OHA alone and BOT groups ($P < 0.05$). As renal dysfunction affects CPR excretion from the kidneys, this could elevate serum FCPR and CPR2h concentrations; therefore, the presence of slight renal dysfunction might have rather underestimated the usefulness of CPR index for MDI, mainly by lowering the sensitivities at the cut-off values of postprandial CPR indices. Incremental CPR by meal load (not included in the present study) could be more useful in patients with renal dysfunction to estimate insulin secretory ability.

In a review, Yagihashi[30] asks, 'What determines the insulin requirement in type 2 diabetes mellitus?' and 'Are all patients who require insulin severely diabetic or in the advanced stage?', thereby advocating the need for clinical staging of type 2 diabetes. It is likely that measurement of some CPR index could be an accurate marker for both setting up the staging and determining the severity of type 2 diabetes, as well as an index to determine a treatment regimen for diabetes.

In conclusion, in patients with non-obese chronic stage type 2 diabetes, postprandial serum CPR value measured at 2 h after breakfast is the optimal CPR index to identify patients requiring MDI. Follow-up evaluation of the selected therapy regimen would confirm the present results; as well as this, another study analyzing CPR indices for MDI therapy in obese type 2 diabetes is required.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1 |** Flow schema from baseline treatment to the final stage therapy.
**Figure S2 |** (a) Receiver operating characteristic analysis of C-peptide immunoreactivity indices for identifying patients who require multiple daily insulin injection using data before intensive insulin therapy. (b) Receiver operating characteristic analysis of C-peptide immunoreactivity indices for identifying patients who require multiple daily insulin injection using data after intensive insulin therapy.
**Table S1 |** (a) Details of oral agents used in patients of the oral hypoglycemic agent alone group. (b) Details of oral agents and insulin used in patients of the basal insulin-supported oral therapy group. (c) Details of insulin used in patients of the multiple daily insulin injection group.
**Table S2 |** (a)Results of multiple logistic analysis of each C-peptide immunoreactivity index (before intensive insulin therapy). (b) Results of multiple logistic analysis of each C-peptide immunoreactivity index (after intensive insulin therapy).
**Table S3 |** Details of renal condition in patients of each therapy group.