Analysis of factors influencing postprandial C-peptide levels in Japanese patients with type 2 diabetes: Comparison with C-peptide levels after glucagon load

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ABSTRACT

Aims/Introduction: Postprandial serum C-peptide levels are readily determined in clinical practice and have a good correlation with serum C-peptide levels after glucagon load; the measurement is often used as an index of endogenous insulin secretion. However, the factors affecting postprandial serum C-peptide levels remain to be evaluated.

Materials and Methods: To investigate the clinical factors affecting postprandial serum C-peptide, 2-h postprandial C-peptide levels after breakfast (PPCPR) were analyzed retrospectively for comparison with glucagon-stimulated C-peptide (CPR-6min) levels measured during hospital admission in 273 Japanese patients with type 2 diabetes.

Results: Multiple regression analysis showed that years from diagnosis, body mass index (BMI) and HbA_{1c} were the major independent variables predicting PPCPR ($R^2 = 0.315$). HbA_{1c} was a major factor predicting PPCPR, but did not predict CPR-6min. In addition, HbA_{1c} was negatively correlated with PPCPR ($r = -0.410$, $P < 0.0001$) and PPCPR/CPR-6min ($r = -0.313$, $P < 0.0001$).

Conclusions: PPCPR was correlated with common factors predicting CPR, including years from diagnosis and BMI, but also was negatively correlated with HbA_{1c}, a unique factor. These results show that chronic elevation of the glucose level might impair endogenous insulin secretion after meal load, but might have little effect on endogenous insulin secretion after glucagon load.

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KEY WORDS: C-peptide, Meal load, HbA_{1c}

INTRODUCTION

Type 2 diabetes is a heterogeneous disease characterized by insulin resistance and defective insulin secretion[1], and is progressive in that the mode of therapy must be altered over the decades of diabetes; diet and exercise therapy alone might be adequate initially, but secondary oral hypoglycemic agent (OHA) treatment and insulin treatment are eventually required[2,3]. This is, at least in part, as a result of progressive loss of pancreatic β-cell function. The results of the United Kingdom Progressive Diabetes Study (UKPDS) show that pancreatic β-cell function (%β), assessed by Homeostasis Model Assessment (HOMA) in patients allocated to diet or OHA decreased approximately 25% in 5 years[4]. In addition, a decline in endogenous insulin secretion over more than several decades of diabetes in patients including insulin-treated patients was observed in a cross-sectional study[5].

Determination of fasting serum C-peptide level and stimulated serum C-peptide level by intravenous glucagon is used widely to assess endogenous insulin secretory reserves[6–9], and the utility of the indices using C-peptide level in choosing insulin therapy has been shown[10]. The postprandial serum C-peptide level can easily be measured in clinical practice and has a good correlation with the serum C-peptide level after glucagon load[11]; it is often used as an index of endogenous insulin secretion, and can be used for both non-insulin-treated and insulin-treated patients[11–13]. Duration of diabetes and body mass index (BMI) are the major factors in serum fasting and glucagon-stimulated C-peptide levels[5,14], but the factors affecting postprandial serum C-peptide levels remain to be evaluated.

In the present study of Japanese patients with type 2 diabetes, to evaluate the clinical factors affecting postprandial serum C-peptide by cross-sectional study, 2-h postprandial C-peptide levels after breakfast were analyzed and compared with glucagon-stimulated C-peptide levels.

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SUBJECTS AND METHODS

Subjects

A total of 388 Japanese patients with type 2 diabetes who were admitted to Kyoto University Hospital between 1997 and 2002 for poor glycemic control were enrolled in the study. Patients with pancreatic or liver disease, taking diabetogenic medications, pregnant or with serum creatinine ≥1.3 mg/dL were excluded from the study. Type 2 diabetes mellitus was diagnosed based on the criteria of the American Diabetes Association (ADA)[15]. Patients with serum creatinine ≥1.3 mg/dL were excluded, as serum C-peptide immunoreactivity (CPR) is elevated by decreased renal function[16]. Of these patients, 115 were excluded as a result of incomplete clinical examinations and the remaining 273 patients, including patients without diabetic medication, oral hypoglycemic agent-treated patients and insulin-treated patients, were analyzed. The clinical profiles of the patients are shown in Table 1.

Methods

On the first day in hospital, medical history, physical examination and laboratory evaluation including glycosylated hemoglobin were carried out. HbA_{1c} was measured using high performance liquid chromatography (HA-8180; Arcray, Kyoto, Japan). The HbA_{1c} (%) value was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent (%) calculated by the formula HbA_{1c} (%) = HbA_{1c} (Japan Diabetes Society [JDS]) (%) + 0.4%, considering the relational expression of HbA_{1c} (JDS) (%) measured by the previous Japanese standard substance and measurement methods and HbA_{1c} (NGSP)[17]. β-Cell function was evaluated within 1 week after an overnight fast by measuring fasting CPR (FCPR), CPR 6min after intravenous injection of 1 mg glucagon (CPR-6min)[6] and postprandial CPR. Serum CPR was measured by radioimmunoassay (Daiichi III; Daiichi Radioisotope Laboratories, Osaka, Japan). Postprandial CPR 2 h after breakfast (PPCPR) was determined. The meal at breakfast was prescribed as nutritional therapy according to the treatment guide for diabetes of the JDS[18], which included 516.6 ± 67.7 kcal (mean ± SD) energy consisting of 49% carbohydrate, 16% protein and 35% fat. In patients taking OHA, medication was stopped for measurement of CPR, but was maintained until 1 day before to prevent hyperglycemia during the test[5]. Plasma glucose was measured by the glucose oxidase method.

The study protocol was approved by the ethics committee of Kyoto University.

Table 1 | Clinical profiles of patients

No. patients	273
Male/female	158/115
Age (years)	61.2 ± 12.2
Years from diagnosis	9.6 ± 9.6
Systolic blood pressure (mmHg)	121.8 ± 12.9
Diastolic blood pressure (mmHg)	73.6 ± 9.6
BMI (kg/m^2)	23.9 ± 3.7
HbA_{1c} at admission (%)	9.7 ± 2.0
sCre (mg/dL)	0.69 ± 0.18
Glucagon load: FPG/PG-6min (mg/dL)	164.1 ± 47.9/180.6 ± 49.1
Glucagon load: FCPR/CPR-6min (ng/mL)	1.80 ± 0.97/3.83 ± 1.76
Meal load: FPG/PPPG (mg/dL)	167.0 ± 54.8/271.5 ± 83.5
Meal load: FCPR/PPCPR (ng/mL)	1.76 ± 0.94/4.87 ± 2.41

BMI, body mass index; CPR-6min, C-peptide immunoreactivity 6 min after intravenous injection of 1 mg glucagon; FCPR, fasting CPR; FPG, fasting plasma glucose; OHA, oral hypoglycemic agents; PG-6min, plasma glucose 6 min after glucagon load; PPCPR, postprandial CPR; PPPG, postprandial plasma glucose; sCre, serum creatinine.

Statistical Analysis

Statistical analysis was carried out with the Stat View 5.0 system (SAS institute Inc., Cary, NC, USA). Data are presented as mean ± SD, unless otherwise noted. The relationship between the parametric clinical data and CPR values was investigated by Pearson's analysis. The relationship between the non-parametric clinical data and CPR values was investigated by Spearman's analysis. Clinical parameters among three groups were compared by analysis of variance (ANOVA). For comparison of two groups, Scheffé's test was carried out. *P*-values < 0.05 were considered statistically significant.

RESULTS

Simple correlation coefficients between FCPR, CPR-6min and PPCPR, and measures of variables (age, years from diagnosis, sex, BMI, systolic and diastolic blood pressure, HbA_{1c}, serum creatinine and plasma glucose [PG]) were calculated and are shown in Table 2. Years from diagnosis and BMI were significantly correlated with all three measures of CPR. PG and HbA_{1c} were significantly correlated with PPCPR ($P < 0.0001$, $r = -0.410$), but not with CPR-6min (Figure 1).

Stepwise multiple regression analysis was carried out using the independent variables in Table 2 to predict CPR as a dependent variable (Table 3). FCPR was independently predicted by years from diagnosis, BMI and serum creatinine, accounting for 22.4% of the variability of FCPR. CPR-6min was independently predicted by years from diagnosis and BMI, accounting for 17.9% of the variability of the dependent variables. PPCPR was independently predicted by years from diagnosis, BMI and HbA_{1c}, accounting for 31.5% of the variability of the dependent variables. Thus, HbA_{1c} is an important independent variable predicting PPCPR, but not FCPR or CPR-6min.

Because HbA_{1c} might be involved in decreased PPCPR, the clinical data among three groups of increased HbA_{1c} (≤8.5%, 8.6–10.3%, ≥10.4%) were compared, as shown in Table 4. Although there was no significant difference among these groups in FCPR and CPR-6min, PPCRP was significantly reduced with increasing levels of HbA_{1c}. CPR-6min was significantly correlated with PPCPR ($P < 0.0001$, $r = 0.564$, PPCPR = 0.774 × CPR-6min + 1.913; Figure 2a). PPCPR was correlated with CPR-6min in each tertile group of HbA_{1c} ($HbA_{1c} \leq 8.5$:

Table 2 | *P*-values and *r*-values of correlation between C-peptide immunoreactivity and measures of variables

	FCPR (ng/mL)	CPR-6min (ng/mL)	PPCPR (ng/mL)
Age (years)	0.4257 (ND)	0.0456 (−0.121)	0.3896 (ND)
Years from diagnosis	0.0024 (−0.182)	<0.0001 (−0.246)	0.0007 (−0.205)
Sex	0.0709 (ND)	0.1879 (ND)	0.8321 (ND)
BMI (kg/m^2)	<0.0001 (0.435)	<0.0001 (0.367)	<0.0001 (0.311)
Systolic blood pressure (mmHg)	0.5551 (ND)	0.9388 (ND)	0.0865 (ND)
Diastolic blood pressure (mmHg)	0.5739 (ND)	0.0327 (0.130)	0.0705 (ND)
HbA_{1c} (%)	0.0443 (−0.122)	0.1507 (ND)	<0.0001 (−0.410)
sCre (mg/dL)	0.0104 (0.155)	0.1641 (ND)	0.0140 (0.148)
FPG (mg/dL)	0.3764 (ND)	ND	ND
PG-6min (mg/dL)	ND	0.7333 (ND)	ND
PPPG (mg/dL)	ND	ND	<0.0001 (−0.285)

All correlations except correlations between sex and C-peptide immunoreactivity (CPR) were analyzed by Pearson's analysis. Correlations between sex and CPR were analyzed by Spearman's analysis. *P*-values are shown. In parenthesis, *r*-values are shown.
BMI, body mass index; CPR-6min, C-peptide immunoreactivity 6 min after intravenous injection of 1 mg glucagon; FCPR, fasting CPR; FPG, fasting plasma glucose; ND, not determined; PG-6min, plasma glucose 6 min after glucagon load; PPCPR: postprandial CPR; PPPG: postprandial plasma glucose; sCre: serum creatinine.

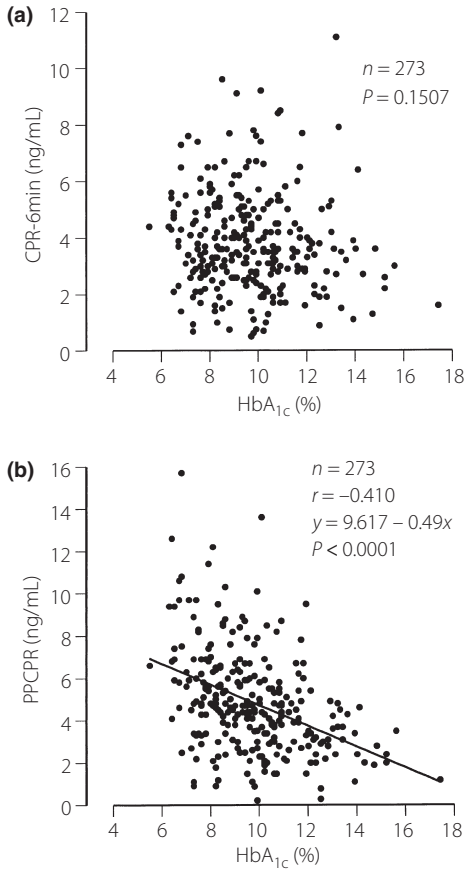

Figure 1 | The relationship between HbA_{1c} and (a) C-peptide immunoreactivity 6 min after intravenous injection of 1 mg glucagon (CPR-6min) and (b) 2-h postprandial C-peptide levels after breakfast (PPCPR).

$P < 0.0001$, $r = 0.595$, $y = 2.159 + 0.970x$, $n = 90$; $8.6\% \leq HbA_{1c} \leq 10.3\%$: $P < 0.0001$, $r = 0.674$, $y = 1.587 + 0.829x$, $n = 92$; $10.4\% \leq HbA_{1c}$: $P < 0.0001$, $r = 0.494$, $y = 2.091 + 0.482x$, $n = 91$). Because the higher HbA_{1c} group was distributed mainly below the regression line of total patients and the lower HbA_{1c} group above the line in the scattergram, and the increase in PPCPR per CPR-6min in the regression line of each tertile group was lower in the higher HbA_{1c} group, we examined the correlation between the ratio of PPCPR to CPR-6min (PPCPR/CPR-6min) and HbA_{1c}. PPCPR/CPR-6min was negatively correlated with HbA_{1c} ($P < 0.0001$, $r = -0.313$; Figure 2b).

DISCUSSION

In the present study, HbA_{1c} was negatively correlated with PPCPR, but not with FCPR or CPR-6min, which suggests that chronic elevation of the glucose level might impair endogenous insulin secretion after a meal load.

Although meal load is not equivalent to glucose load, as it contains nutrients other than carbohydrates that modulate glucose-induced insulin secretion, elevated glucose in plasma might play an important role in meal-stimulated insulin secretion. Indeed, the plasma glucose level after a meal load was increased considerably to more than 100 mg/dL in average. In contrast, the increment of glucose after glucagon load was only approximately 15 mg/dL, indicating a small contribution of glucose elevation to increased insulin secretion by glucagon loading.

Because HbA_{1c} was positively correlated with PPPG in the present study ($P < 0.0001$, $r = 0.570$), HbA_{1c} reflects postprandial glucose level. In simple correlation, both HbA_{1c} and PPPG were significantly correlated with PPCPR; whereas in stepwise regression analysis, HbA_{1c} was important to predict PPCPR, but PPPG was not. In addition, in simple correlation to PPCPR, the *r*-value for HbA_{1c} (0.410) was larger compared with that for PPPG (0.285; Table 2). These results show that PPCPR is more strongly affected by chronic elevation of glucose levels than by transient elevation of glucose levels.

Multiple regression analysis showed that years from diagnosis, BMI and HbA_{1c} were the major independent variables predicting PPCPR. This shows that years from diagnosis and BMI are common major factors predicting CPR. In contrast, HbA_{1c} was the major factor predicting PPCPR, but not FCPR or CPR-6min, and was negatively correlated with PPCPR. We hypothesized that CPR-6min reflects reserve capacity of endogenous insulin secretion independent of glycemic control and that PPCPR is predicted by a fundamental factor independent of glycemic control and by a variable factor dependent of glycemic control. CPR-6min predicted 31.8% of the variability of PPCPR as shown in Figure 2a. When a regression model using CPR-6min

Table 3 | Stepwise multiple regression analysis for predictors of C-peptide immunoreactivity

	F-value	Partial regression coefficient	Standard partial regression coefficient	R^2 (R)
FCPR (ng/mL)				
Years from diagnosis	9.4	−0.017	−0.170	0.224 (0.473)
BMI (kg/m^2)	55.2	0.108	0.406	
sCre (mg/dL)	7.3	0.823	0.149	
CPR-6min (ng/mL)				
Years from diagnosis	14.6	−0.039	−0.214	0.179 (0.423)
BMI (kg/m^2)	38.9	0.170	0.349	
PPCPR (ng/mL)				
Years from diagnosis	23.4	−0.063	−0.252	0.315 (0.561)
BMI (kg/m^2)	27.5	0.178	0.270	
HbA_{1c} (%)	68.7	−0.516	−0.431	

BMI, body mass index; CPR-6min, C-peptide immunoreactivity 6 min after intravenous injection of 1 mg glucagon; FCPR, fasting CPR; PPCPR, postprandial CPR; sCre, serum creatinine.

Table 4 | Comparison of clinical characteristics and clinical profile among groups according to HbA_{1c} at admission

Groups (HbA_{1c} at admission)	I (≤8.5%)	II (8.6–10.3%)	III (≥10.4%)	*P*
No. patients	90	92	91	
HbA_{1c} (%)	7.6 ± 0.1	9.5 ± 0.1*	12.0 ± 0.1*†	<0.0001
Sex (male/female)	53/37	55/37	50/41	
Age (years)	64.2 ± 1.2	61.6 ± 1.3	57.6 ± 1.3*	0.0011
BMI (kg/m^2)	24.2 ± 0.3	24.1 ± 0.4	23.5 ± 0.4	0.3579
Years from diagnosis	11.7 ± 1.2	9.7 ± 0.8	7.4 ± 0.8*	0.0088
bSBP (mmHg)	122.9 ± 1.4	120.6 ± 1.3	121.3 ± 1.4	0.4746
DBP (mmHg)	72.9 ± 1.2	72.7 ± 1.0	75.3 ± 0.9	0.1302
sCre (mg/dL)	0.74 ± 0.02	0.70 ± 0.02	0.63 ± 0.02*†	<0.0001
FPG (mg/dL)	134.2 ± 3.7	163.4 ± 3.9*	195.0 ± 5.4*†	<0.0001
PG-6min (mg/dL)	152.4 ± 3.9	178.7 ± 4.0*	211.1 ± 5.6*†	<0.0001
PPPG (mg/dL)	223.3 ± 6.4	268.2 ± 7.7*	323.7 ± 8.9*†	<0.0001
FCPR (ng/mL)	1.92 ± 0.10	1.84 ± 0.10	1.66 ± 0.11	0.2004
CPR-6min (ng/mL)	3.85 ± 0.18	3.97 ± 0.19	3.66 ± 0.18	0.5467
PPCPR (ng/mL)	5.90 ± 0.29	4.88 ± 0.24*	3.86 ± 0.18*†	<0.0001

Data are presented as mean ± SE.
*$P < 0.01$ vs group I, †$P < 0.01$ vs group II.
BMI, body mass index; CPR-6min, C-peptide immunoreactivity 6 min after intravenous injection of 1 mg glucagon; DBP, diastolic blood pressure; FCPR, fasting CPR; FPG, fasting plasma glucose; PG-6min, plasma glucose 6 min after glucagon load; PPCPR, postprandial CPR; PPPG, postprandial plasma glucose; SBP, systolic blood pressure; sCre, serum creatinine. FPG and FCPR are values when meal load was carried out.

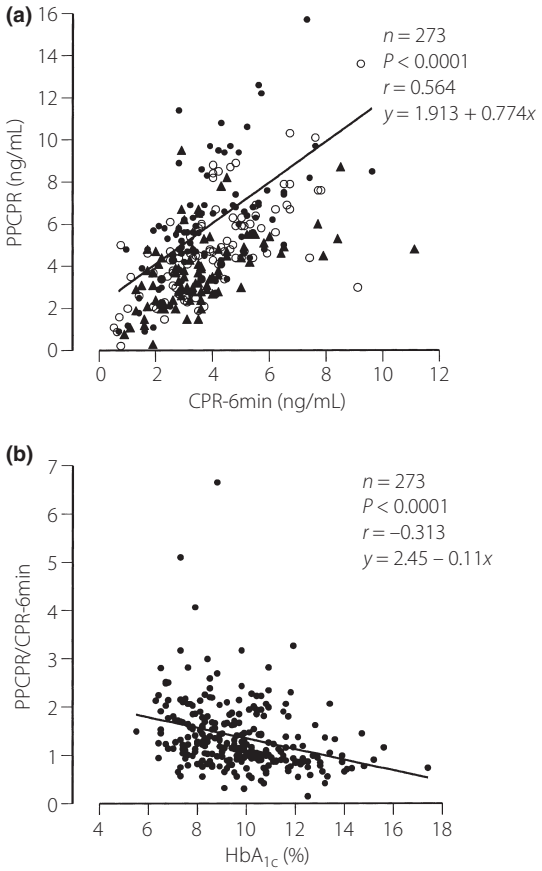

Figure 2 | Relationship between (a) C-peptide immunoreactivity 6 min after intravenous injection of 1 mg glucagon (CPR-6min) and 2-h postprandial C-peptide levels after breakfast (PPCPR) and (b) PPCPR/CPR-6min and HbA_{1c}. Black circles, $HbA_{1c} \leq 8.5\%$; white circles, $8.6\% \leq HbA_{1c} \leq 10.3\%$, black triangles, $10.4\% \leq HbA_{1c}$

and HbA_{1c} as independent variables to predict PPCPR as a dependent variable was used, CPR-6min and HbA_{1c} predicted 44.9% of the variability of PPCPR ($P < 0.0001$, $R = 0.670$, $PPCPR = 6.286 + 0.730 \times CPR\text{-}6min - 0.434 \times HbA_{1c}$). The addition of HbA_{1c} as an independent variable increased the prediction of the variability of PPCPR by 13.1%. In the present study, PPCPR/CPR-6min was used as a putative index of variability dependent of glycemic control and was found to be correlated with HbA_{1c} in the present study (Figure 2b). Furthermore, improvement of glycemic control by treatment ameliorates the CPR response after oral glucose load[19–21]. In addition, the CPR response after glucagon load is affected little by treatment to improve hyperglycemia and it is not correlated with the CPR response after oral glucose load before treatment, whereas it is well-correlated with improved CPR response after oral glucose load after treatment[21]. Reversible impairment of endogenous insulin response after glucose load is explained by glucose toxicity, in which chronic hyperglycemia deteriorates meal-induced and glucose-induced insulin secretion and insulin-sensitive glucose disposal[22]. Therefore, the chronic high glucose

level shown by high HbA_{1c} might impair endogenous insulin secretion after meal load, but has little effect on endogenous insulin secretion after glucagon load. The lack of influence of HbA_{1c} on CPR-6min might be helpful to evaluate reserve capacity of endogenous insulin secretion, even when glycemic control is poor enough to deteriorate postprandial insulin secretion. In contrast, PPCPR is affected by HbA_{1c} and might reflect the state of deteriorated insulin secretion by glucose toxicity that may be recovered by improved glycemic control.

In stepwise regression analysis, HbA_{1c} was not important to predict FCPR, but was important to predict PPCPR. In simple correlation, HbA_{1c} was significantly negatively correlated not only with PPCPR, but also with FCPR, whereas the *P*-value and *r*-value for FCPR were larger and smaller, respectively, compared with those for PPCPR (Table 2). Taken together, these findings suggest that glucose toxicity might deteriorate not only postprandial insulin secretion, but also fasting insulin secretion, whereas postprandial insulin secretion might be more vulnerable to glucose toxicity than to fasting insulin secretion.

The suppressive effect of glucose toxicity on insulin secretion *in vivo* might be attributable to impairment of β-cell responsiveness to glucose[22] and to impairment of incretin effect[23,24]. However, it is important to understand why glucagon-stimulated CPR is preserved despite severe impairment of glucose-stimulated CPR before treatment to improve hyperglycemia[21]. This remains largely unknown, but our hypothesis based on an *in vitro* study is that deteriorated intracellular glucose metabolism plays an important role in impaired glucose-induced insulin secretion[25] and that increased intracellular cyclic adenosine 3′,5′-monophosphate concentration derived from glucagon stimulation ameliorates impaired intracellular glucose metabolism to improve suppressed insulin secretion[26].

A recent study showed that indices using CPR correlate well with β-cell mass by analysis of β-cell areas of samples obtained during pancreatectomy and serum levels of CPR before operation[27]. Thus, PPCPR might reflect not only β-cell mass, but also reversible impairment of endogenous secretion as a result of chronic glucose elevation.

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The authors declare no conflict of interest.

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