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Early-phase insulin secretion is disturbed in obese subjects with glucose intolerance

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Abstract

The loss of early-phase insulin secretion is a characteristic feature of type 2 diabetes mellitus. The aim of this study is to examine when impairment of early-phase insulin secretion occurs and whether it can be related to increase in insulin resistance caused by obesity. We developed an analytical method to qualify the early-phase insulin secretion; that is, we measured C-peptide immunoreactivity (CPR) response to a selective increase in blood glucose level in portal vein during oral glucose load under a euglycemic hyperinsulinemic clamp (clamp-OGL). Glucose infusion rate, hepatic glucose uptake, and CPR response during clamp-OGL were measured in 30 subjects with diabetes who were divided into 3 groups based on body mass index, 13 obese subjects with normal glucose tolerance (O-NGT), 10 obese subjects with impaired glucose tolerance (O-IGT), and 15 healthy subjects. Significant increase in CPR levels at 10 minutes in clamp-OGL compared with those at steady state was observed in healthy subjects and in O-NGT; however, those were small or absent in diabetic patients and in O-IGT. The incremental ratio of CPR was not correlated to the makers of insulin resistance. The early-phase insulin secretion is well maintained in O-NGT; however, early-phase insulin secretion has already been disturbed in obese subjects with glucose intolerance.

1. Introduction

In humans, insulin resistance leads to type 2 diabetes mellitus (DM), the nature of which varies widely. The clinical features associated with type 2 DM represent a complex disease [1-3]. For example, some patients with type 2 DM have a mild form of the disease for a long period, whereas others become progressively worse and develop severe diabetes. The causes of the different manifestations of type 2 DM are unclear.

It is well known that early-phase insulin secretion is impaired in patients with type 2 DM; however, what causes this impairment is unclear. It has recently been reported that the hepatoportal glucose sensor may be important in the regulation of glucose homeostasis; however, only a limited number of animal studies have been published [4,5], and no reports on humans are available because of the lack of quantitative analytical methods to evaluate this process in vivo.

The euglycemic clamp technique [6] is usually used in conjunction with routine examinations to evaluate insulin sensitivity and insulin secretion in humans. This technique, however, cannot be used to evaluate glucose uptake in the liver, which is the regulatory center of blood glucose levels [7]. Kawamori et al [8] developed a new technique that allows the use of an oral glucose load (OGL) under a euglycemic hyperinsulinemic clamp (clamp-OGL) to examine hepatic glucose uptake (HGU) as a marker of hepatic insulin sensitivity because of the inability of using tracer method in Japan. During hyperinsulinemic euglycemic clamp, the plasma glucose (PG) level could not stimulate the glucose sensor of pancreatic beta cells or cephalic phase because the PG level was maintained at a euglycemic level (5.0 mmol/L), whereas the portal glucose level could be elevated in clamp-OGL. High concentrations of exogenous insulin would be expected to suppress the endogenous insulin secretion from pancreatic beta cells. We noticed that the serum C-peptide immunoreactivity (CPR) levels were

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altered during the clamp-OGL and measured the CPR response at 10 minutes during OGL-clamp.

In this study, we examined whether impediment of CPR response to clamp-OGL had already been seen in patients with early type 2 DM and nondiabetic obese subjects and whether the extent of hindrance of early-phase insulin secretion could be related to increase in insulin resistance.

2. Methods

2.1. Subjects

The study included 68 subjects: 30 patients with type 2 DM, 10 obese subjects with impaired glucose tolerance (O-IGT), 13 obese subjects with normal glucose tolerance (O-NGT), and 15 healthy subjects. The definitions of DM, O-IGT, and O-NGT were based on World Health Organization criteria [9]. The protocol for the study was received and approved by the human subjects review committee of the University of Tokushima (Tokushima, Japan) and was conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent before participation in our study.

An oral glucose tolerance test (OGTT) was conducted on all subjects before the OGL-clamp study. The 30 patients with type 2 DM were divided into 3 groups according to BMI (group 1, BMI $\geq$25.0 kg/m^2 [overweight group]; group 2, BMI >20.0 and <25.0 kg/m^2 [normal body weight group]; and group 3, BMI $\leq$20.0 kg/m^2 [underweight group]). Patients were not divided according to glycemic control, type of therapy, or duration of diabetes. The criterion of obesity in Japan is more than 25.0 of BMI, so we simply divided the subjects into groups by their BMI. Diabetic patients in group 1 with BMI of 25.0 kg/m^2 or higher, in whom diabetic duration was from 1 to 3 years, were treated with diet therapy, or glinide and/or metformin. Diabetic patients in group 2 with BMI of 20 to 25 kg/m^2, in whom diabetic duration was more than 3 years, were treated with sulfonylurea and/or pioglitazone. Diabetic patients in group 3 with BMI of 20.0 kg/m^2 or less, in whom diabetic duration were more than 7 years, were treated with sulfonylurea or insulin. The 20 obese subjects were also divided into 2 groups (O-IGT and O-NGT) to examine if any differences existed on the early-phase insulin secretion in obese subjects with or without IGT. The clinical characteristics of all study subjects are shown in Table 1.

2.2. Glucose clamp study

Glucose infusion rate (GIR), HGU, and the serum CPR levels during clamp-OGL were determined. Subjects were admitted to our hospital at 8:00 AM after an overnight fast. They voided, were weighed, and then remained supine for the duration of the procedure. A polyethylene catheter was inserted into an antecubital vein in a retrograde manner for the administration of glucose (20% of glucose) and insulin infusion. A second catheter was inserted into a dorsal hand vein on the contralateral arm in a retrograde fashion and kept in a warming device to arterialize the venous blood. Blood samples were drawn at baseline to determine fasting PG (FPG) levels, serum immunoreactive insulin (IRI) levels, CPR levels, and glycated hemoglobin (HbA_{1c}) values. Insulin-mediated whole-body glucose uptake was measured using an artificial pancreas (Model STG-22 Nikkiso, Tokyo, Japan), according to the method of DeFronzo et al [6]. An infusion of 20% of glucose solution was started at baseline, and the rate was adjusted to clamp the glucose level at 5.0 mmol/L during the clamp study. Blood glucose levels were checked at 10-second intervals throughout the investigation. Data on total-body glucose uptake represent the mean values for the GIR during the final 30 minutes of infusion. Glucose was orally administrated at a dose of 0.2 g/kg of body weight after determining the baseline GIR during the hyperinsulinemic euglycemic clamp described above. Thereafter, the hyperinsulinemic euglycemic clamp was continued and the extent of decrease in GIR was monitored for 120 minutes to evaluate HGU, which was used as a parameter of insulin sensitivity in the liver. Hepatic glucose uptake was calculated according to the method of Kawamori et al [8] and expressed as a percentage of total loading of glucose. Serum CPR levels were measured at 0 hour (before clamp), 2 hours (0 minute in clamp-OGL), and 10 and 60 minutes after an OGL-clamp study. The schematic procedure of OGL-clamp is shown in Fig. 1.

Table 1
Clinical characteristics of study subjects

	Diabetic patients			Obese subjects		Healthy subjects
	Group 1	Group 2	Group 3	O-IGT	O-NGT	
n	10	10	10	10	13	15
Age (y)	46 ± 9	52 ± 13	57 ± 6	46 ± 9	44 ± 14	33 ± 11
Sex (F/M)	2/8	3/7	3/7	3/7	5/7	5/10
BMI (kg/m^2)	29.1 ± 2.4^{a,d}	23.1 ± 2.0^{d,f}	19.5 ± 0.8	28.6 ± 1.9	28.2 ± 2.4	21.8 ± 1.8
FPG (mmol/L)	8.7 ± 1.6^{c,e,f}	10.2 ± 1.9^{f}	11.8 ± 2.4	6.4 ± 0.4	5.9 ± 0.3	4.8 ± 0.3
HbA_{1c} (%)	7.3 ± 0.7	8.5 ± 1.4	8.4 ± 1.1	5.8 ± 0.2	5.5 ± 0.2	4.8 ± 0.2
IRI (pmol/L)	146 ± 41^{b,d,g}	76 ± 45^{e}	33 ± 12	101 ± 14	90 ± 14	41 ± 24
CPR (pmol/L)	948 ± 331^{a,d,h}	327 ± 133^{c}	221 ± 65	723 ± 96	631 ± 69	352 ± 54
HOMA-R (index)	8.3 ± 4.1^{a,d,f}	5.5 ± 2.8^{c}	3.2 ± 2.2	3.7 ± 0.7	4.4 ± 1.0	1.1 ± 0.4

$^{a}P < .001$, $^{b}P < .01$, and $^{c}P < .05$ vs group 2; $^{d}P < .001$ and $^{e}P < .05$ vs group 3; $^{f}P < .001$, $^{g}P < .01$, and $^{h}P < .05$ vs obese subjects.

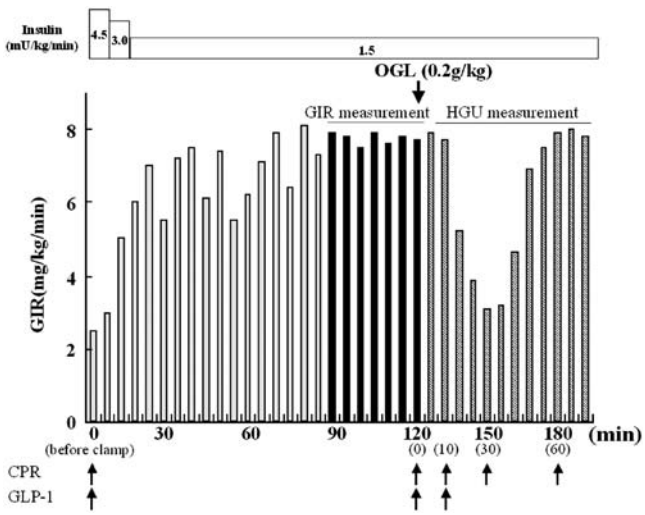

Fig. 1. Procedure of evaluation of CPR response to an oral glucose load during clamp-OGL.

2.3. Laboratory analysis

Immunoreactive insulin and CPR were measured by a commercially available radioimmunoassay kit (EIKEN, Tokyo, Japan); plasma active glucagon-like peptide 1 (active GLP-1) was measured by an enzyme-linked immunosorbent assay kit (Linco, MO), and PG was measured by the glucose oxidase method. Glycated hemoglobin was determined by high-performance liquid chromatography, and serum total cholesterol, triglyceride, and high-density lipoprotein cholesterol levels were determined by an enzymatic technique using a model 736 HITACHI autoanalyzer (Mito, Japan). The insulinogenic index is calculated as the ratio between increases in the concentration of plasma insulin during the first 30 minutes after an OGTT and the concentration of PG over the same period (ΔIRI30/ΔPG30). Homeostasis model assessment insulin resistance index (HOMA-R) was calculated according to the formula of Matthews et al [10].

2.4. Statistical analysis

Data are expressed as mean ± SD unless otherwise specified. The statistical significance of differences was analyzed using the analysis of variance, followed by Student *t* test for individual comparison of mean values. Correlations were evaluated using multivariate analysis. A software package for Macintosh (Stat View 4.11, Abacus Concepts, Berkeley, CA) was used for the statistical analyses. A *P* value of less than .05 was considered to be statistically significant.

3. Results

3.1. C-peptide immunoreactivity response at 10 minutes after OGL and incremental ratio of CPR during OGL-clamp

Changes in CPR levels during clamp-OGL are shown in Fig. 2. The basal CPR levels (before clamp) in obese subjects (group 1, O-IGT, and O-NGT) were high and those

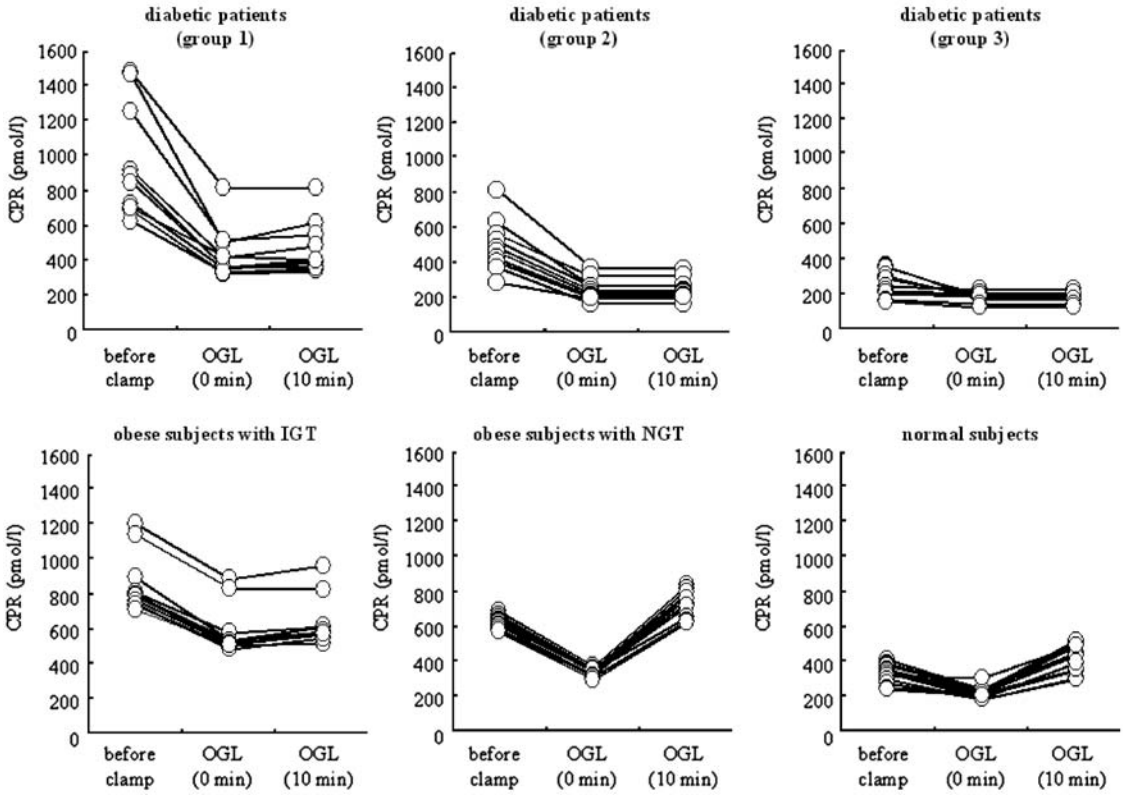

Fig. 2. Changes in serum CPR levels during OGL-clamp at baseline (before clamp), steady state (0 minute), and 10 minutes in patients with diabetes in group 1 (BMI ≥25.0 kg/m^2), group 2 (BMI >20.0 and <25.0 kg/m^2), and group 3 (BMI ≤20.0 kg/m^2), and in O-IGT, O-NGT, and healthy subjects.

Table 2
Glucose infusion rate, HGU, insulinogenic index, and IRC in all subjects

	GIR (mmol/min)	HGU (%)	Insulinogenic index (AU)	IRC (AU)
Healthy subjects	43.4 ± 5.7	53.8 ± 3.0	0.89 ± 0.19	0.90 ± 0.35
O-NGT	25.3 ± 3.8[a]	40.1 ± 5.2[a]	0.59 ± 0.17[a]	0.92 ± 0.27
O-IGT	15.5 ± 3.1[a,b]	32.7 ± 5.4[a,b]	0.34 ± 0.10[a,c]	0.13 ± 0.08[a,c]
DP group 1	18.5 ± 5.0[a]	31.1 ± 4.8[a,d]	0.28 ± 0.07[a,d]	0.15 ± 0.11[a,b,d]
DP group 2	21.7 ± 6.2[a,d]	34.7 ± 11.5[a,d]	0.16 ± 0.06[a,b]	0.05 ± 0.07[a,b]
DP group 3	42.2 ± 3.6	54.9 ± 4.9	0.09 ± 0.03[a,b]	0.01 ± 0.03[a,b]

[a]$P < .001$ vs healthy subjects; [b]$P < .01$ and [c]$P < .001$ vs O-NGT; [d]$P < .001$ vs group 3. AU indicates arbitrary unit; DP, diabetic patient.

in nonobese subjects (groups 2, 3, and healthy subjects) were low. The CPR levels at 0 minute in clamp-OGL decreased compared with those at basal levels. The increase in CPR levels at 10 minutes was small or absent in the patients in group 1 and in O-IGT and absent in the patients in groups 2 and 3. However, the CPR levels at 10 minutes were increased in O-NGT and healthy subjects. The incremental ratio of CPR (IRC) was calculated using the formula: [CPR (10 minutes) − CPR (0 minute)]/CPR (0 minute), to evaluate early-phase insulin secretion more efficiently than using CPR values at 10 minutes, as shown in Table 2. The IRC in all patients with DM and O-IGT were significantly lower than those in healthy subjects and in O-NGT ($P < .001$). Similarly, the values for patients in group 1 and O-IGT were significantly higher than those in groups 2 and 3 ($P < .05$). The GIR and HGU in O-IGT and in the patients in group 1 were significantly lower than those in O-NGT, and those were not different between the O-IGT group and in patients in group 1, as shown in Table 2. Plasma active GLP-1 levels were measured in all subjects in the study, but these levels did not increase at 10 minutes after the OGL during the clamp study, compared with values at baseline and at the steady state (data not shown).

3.2. Changes in PG and IRI levels during the OGTT

Changes in PG and IRI levels during the OGTT are shown in Fig. 3. Peak PG concentrations were 10.5 ± 2.8 mmol/L in O-NGT, 12.1 ± 2.0 mmol/L in O-IGT, 16.4 ± 2.7 mmol/L in group 1, 16.8 ± 2.2 mmol/L in group 2, and 17.6 ± 3.5 mmol/L in group 3, respectively. Immunoreactive insulin concentrations 30 minutes after an OGTT were 522.1 ± 107.3 pmol/L in O-NGT, 272.3 ± 94.1 pmol/L in O-IGT, 346.2 ± 72.0 pmol/L in group 1, 210.3 ± 78.1 pmol/L in group 2, and 118.9 ± 21.7 pmol/L in group 3,

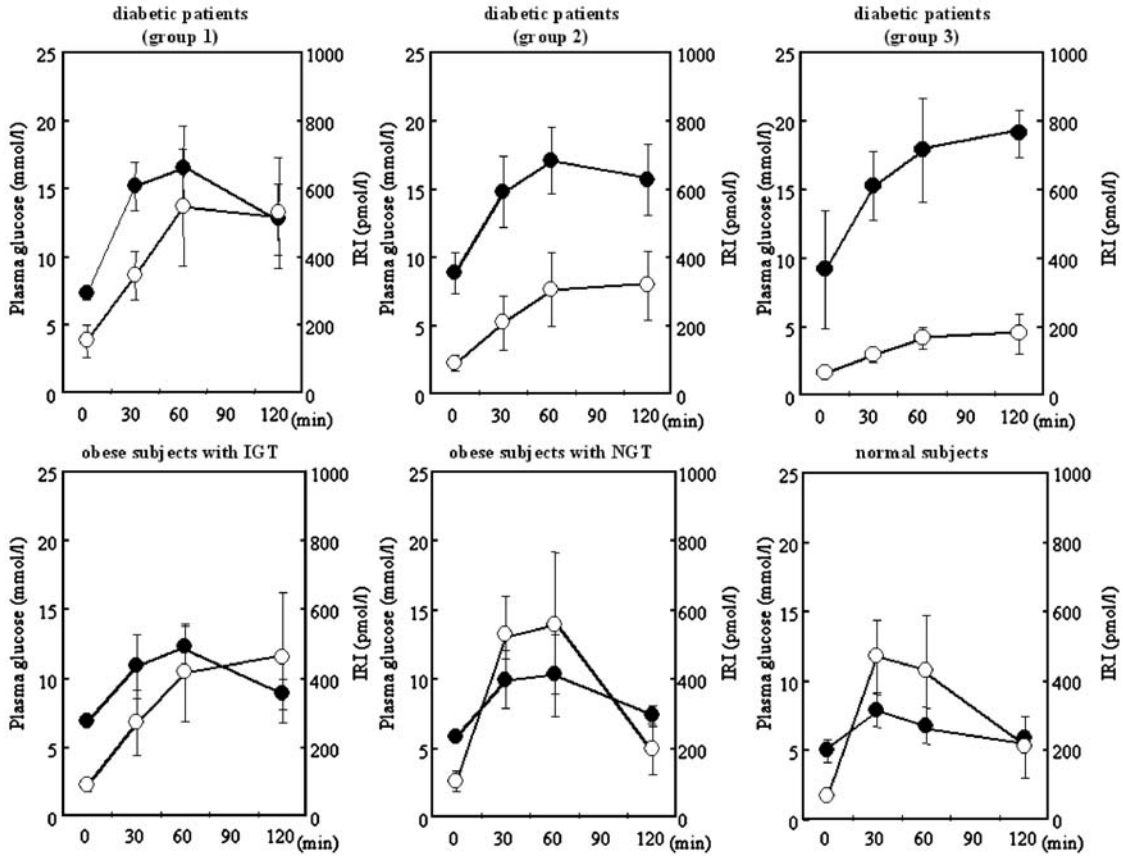

Fig. 3. Changes in PG and serum IRI levels during a 75-g OGTT in patients with diabetes in groups 1, 2, and 3, and in O-IGT, O-NGT, and healthy subjects.

Table 3
Relationship of CPR-related parameters to GIR, HGU, HOMA-R, BMI, FPG, and HbA_{1c} in all subjects

	GIR	HGU	HOMA-R	BMI	FPG	HbA_{1c}
CPR (basal level)	−0.75[a]	−0.69[a]	0.69[a]	0.78[a]	−0.09	0.04
CPR (OGL 0 min)	−0.63	−0.58[b]	0.55[b]	0.81[a]	−0.13	−0.18
CPR (OGL 10 min)	−0.28	−0.22	0.20	0.74[a]	−0.64[b]	−0.60[b]
IRC at 10 min	0.29	0.28	−0.26	0.11	−0.79[a]	−0.81[a]

[a] $P < .001$, [b] $P < .01$.

respectively. Immunoreactive insulin response at 30 minutes in O-IGT was significantly reduced compared with those in O-NGT. Similarly, the insulinogenic index by the OGTT in O-IGT was significantly lower than that in O-NGT ($P < .001$) as shown in Table 2.

3.3. Relationship of CPR-related parameters to metabolic parameters

The coefficient of correlation for CPR levels and other parameters is shown in Table 3. Serum CPR levels at baseline were significantly correlated with GIR, HGU, HOMA-R, and BMI as a marker of insulin resistance not but with FPG and HbA_{1c} as a marker of glycemic control, which related to insulin secretability. Conversely, the IRC was significantly correlated with FPG and HbA_{1c} but not with GIR, HGU, HOMA-R, and BMI. Furthermore, serum CPR levels at 0 and 10 minutes were well correlated only with BMI.

4. Discussion

In this study, an attempt was made to examine whether the CPR response to OGL under hyperinsulinemic euglycemic conditions was parallel to the decrease in early-phase insulin secretion and was related with the extent of insulin resistance. The loss of early-phase insulin secretion has been commonly observed in the patients with overt type 2 DM [11]. It is well known that the hepatoportal glucose sensor is important in glucose homeostasis because of its ability to regulate hepatic glucose production and uptake [12] and stimulate sympathoadrenal counterregulation to hypoglycemia [13]. The increase in CPR levels at 10 minutes in clamp-OGL reflects the glucose sensing in the portal vein not but the response of pancreatic beta cells or changes in the cephalic phase, as the PG level was maintained at a euglycemic level (5.0 mmol/L) and high amounts of exogenous insulin were capable of suppressing endogenous insulin secretion from the beta cells during OGL-clamp. Consequently, the decrease and absence of CPR response to clamp-OGL could reflect the disorders in the glucose sensor in the hepatoportal region or the secretory defect of pancreatic beta cells, which could not react to the signals mediated by the hepatoportal glucose sensor.

The increase in CPR levels at 10 minutes in clamp-OGL was either small or absent in obese patients with early type 2 DM and in O-IGT, compared with that in healthy subjects as well as in O-NGT. It was particularly noteworthy that the CPR response at 10 minutes in clamp-OGL was already disturbed in O-IGT but not in O-NGT who showed insulin resistance. In addition, the peak PG concentrations at 2 hours by the OGTT were more than 9.0 mmol/L in 3 patients from the O-IGT group who showed no response in CPR levels by OGL-clamp; truly, these 3 patients developed overt diabetes within 1 year. Furthermore, we have been following up the subjects for 3.5 years; 5 obese subjects with IGT including 3 subjects mentioned above showing low ICR had an onset of type 2 DM within 2 years; on the contrary, 13 obese subjects with NGT showing good ICR did not develop diabetes. It is suggested that the O-NGT group, who showed a significant increase in CPR levels at 10 minutes, would not develop DM within a few years and the O-IGT group, who showed a remarkable decrease in CPR at 10 minutes, would develop DM in the future. The early-phase insulin secretion is commonly assessed by an intravenous glucose tolerance test [14,15] or insulinogenic index by the OGTT [16]. The OGTT evaluates the function of the glucose sensor in the cephalic phase, portal vein, and pancreatic beta cells as well as the ability of insulin secretion in the pancreatic beta cells. The data from the clamp-OGL study would demonstrate that pancreatic beta cells had already been disturbed in patients at a prediabetic stage and gradually began worsening after patients developed diabetes. Furthermore, the IRC in clamp-OGL was strongly correlated with the insulinogenic index by the OGTT, which is usually used to determine hindrance of early-phase insulin secretion. It is difficult to distinguish mild deterioration of it in O-NGT from that in O-IGT and patients with early type 2 DM with the insulinogenic index by the OGTT. The IRC in clamp-OGL could completely distinguish between the O-NGT and O-IGT groups and obese patients with diabetes (group 1) in our study. This finding indicates that evaluation of the CPR response in clamp-OGL was superior to insulinogenic index during the OGTT to detect a disturbance in early-phase insulin secretion in vivo. It is indicated that the decrease of ICR in clamp-OGL might more exactly detect the defect of early-phase insulin secretion in the subjects at the early stage of glucose intolerance than other methods.

It is necessary to consider the enteroinsular axis [17,18], which may influence early-phase insulin secretion, as assessed by the OGL-clamp method. Several studies have reported that incretin action is usually disturbed in patients with type 2 DM [19,20]. Plasma active GLP-1 levels in all subjects did not increase at 10 minutes after the OGL during

the clamp study compared with values at baseline and at steady state. Nakabayashi et al [21] reported that GLP-1 was more closely related to glucose sensing in the hepatoportal region than GIP. Furthermore, active GLP-1 did not increase at 10 minutes during clamp-OGL; however, it was reported that intraportal infusion of glucose and active GLP-1 augmented insulin release [22]. Therefore, the increase in CPR at 10 minutes in the OGL-clamp may not be influenced by incretin.

In the clamp-OGL study, we measured CPR concentrations at baseline, steady state, and 10 minutes after the OGL. The basal level of CPR in patients with diabetes in group 1 was significantly higher than that in nondiabetic obese subjects with or without IGT and other patients with diabetes. This elevation in patients in group 1 might be induced by fasting hyperglycemia because of the ability of these patients to maintain basal insulin secretion. The basal CPR concentration was well correlated with GIR, HGU, HOMA-R, and BMI, which are parameters of insulin resistance, but not with FPG and HbA_{1c} levels, which are parameters of glycemic control regulated by insulin secretion (Table 3). The IRC at 10 minutes was well correlated with FPG and HbA_{1c} levels, not but with GIR, HGU, HOMA-R, and BMI. These findings indicate that the basal CPR levels reflect insulin resistance and that the IRC at 10 minutes reflects the ability of insulin secretion.

Then, insulin-mediated whole-body glucose disposal represented by the GIR was well correlated with HGU as a parameter of insulin sensitivity in the liver [23]. This finding indicates that insulin resistance coexists in the peripheral tissue and the liver tissue. Nevertheless, the patients with type 2 DM in our experiment did not always experience insulin resistance, as the GIR and HGU in all 10 patients with diabetes in group 3 (lean patients) remained normal. Lean diabetic patients with a defect in insulin secretion may not experience insulin resistance, although their FPG levels were more than 10 mmol/L. This result is supported by clinical evidence that the patients with type 2 DM with abdominal obesity displayed peripheral insulin resistance in combination with defective insulin secretion, whereas nonobese diabetic patients showed only a secretory defect [24].

We have to discuss which mechanism would induce the disturbance in early-phase insulin secretion. Previous studies have reported that the glucoreceptors in hepatoportal lesion, which presumably consists of nerves [25,26], have shown that they can affect insulin release from pancreatic islet [27], and stimulation from them decreases activity in afferent vagus nerve fibers that project on vagal nuclei in the brain stem [28]. Furthermore, nerves in the adventitia of the hepatic artery, that is, mainly sympathetic nerve, may transfer the signals from the liver to islets, affecting insulin release [29,30]. Thereafter, any neuropathy can modulate early-phase insulin secretion. Our findings show that hindrance of early-phase insulin secretion has already occurred in prediabetic O-IGT.

In conclusion, we have found that early-phase insulin secretion was disturbed in obese patients with early type 2 DM and O-IGT not but in O-NGT. This deterioration in insulin secretion provides common pathogenic evidence for the patients with type 2 DM and is not related to insulin resistance.

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