

# Lack of C-Peptide Suppression by Exogenous Hyperinsulinemia in Subjects with Symptoms Suggesting Reactive Hypoglycemia

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**Abstract.** The C-peptide suppression test employing the euglycemic hyperinsulinemic clamp technique has been proposed as a useful diagnostic measure for insulinoma. To examine the specificity of the C-peptide suppression, we applied this test to subjects with symptoms suggesting reactive hypoglycemia. Five subjects studied had never experienced fasting hypoglycemia, and were negative in ultrasound, CT and MRI of the pancreas. Plasma C-peptide was not suppressed by physiological (50–100  $\mu\text{U}/\text{ml}$ ) and supraphysiological (200–500  $\mu\text{U}/\text{ml}$ ) hyperinsulinemia (% of baseline:  $97.3 \pm 8.6\%$  and  $90.6 \pm 10.4\%$ ,  $\pm$  SEM, respectively, both NS). Three subjects were re-examined one year later, when their hypoglycemic episodes were noticeably attenuated. No significant suppression was found. Significant suppression was observed when plasma glucose was clamped at 50–60 mg/dl in four of five subjects ( $61.7 \pm 11.5\%$ ,  $P < 0.05$ ), but one subject responded to neither higher plasma insulin nor low-normal glucose. In contrast, normal glucose tolerance ( $n=13$ ), IGT ( $n=12$ ) and obese NIDDM ( $n=31$ ) subjects showed highly significant suppression during euglycemic and physiological hyperinsulinemia ( $37.1 \pm 3.8\%$ ,  $46.3 \pm 5.6\%$ ,  $39.9 \pm 2.6\%$ , respectively, all  $P < 0.001$ ). In conclusion, the results of the present study indicate that a failure of hyperinsulinemic suppression of C-peptide in euglycemia is not specific for insulinoma, and that suppression of C-peptide by insulin at lower plasma glucose levels (50–60 mg/dl) would be a better diagnostic test.

**Key words:** Reactive hypoglycemia, Hyperinsulinemic glucose clamp, C-peptide suppression test  
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A VARIETY of biochemical tests for the diagnosis of insulinoma largely depend on the inappropriate secretion of insulin to the prevailing plasma glucose level, but it can be difficult to detect a definite imbalance between plasma insulin and glucose, even after prolonged fasting. The C-peptide suppression test has been proposed as a reliable diagnostic technique [1–5], which is based on the assumption that hyperinsulinemia in the presence of normoglycemia inhibits the secretion of insulin

by normal pancreatic B-cells but not by neoplastic B-cells. To investigate whether this assumption is true, the present study addressed insulin regulation of B-cell function in subjects with symptoms suggesting reactive hypoglycemia.

## Subjects and Methods

### Subjects

The present study included five patients who were referred to our Diabetes Center with symptoms suggesting reactive hypoglycemia. Clinical features of the subjects are shown in Table 1. Hypoglycemia was documented in three subjects (36,

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**Table 1.** Clinical features of the subjects with reactive hypoglycemia

	Case 1	Case 2	Case 3	Case 4	Case 5
Age (year), Sex	25, F	16, F	47, M	66, M	41, F
BMI (kg/m <sup>2</sup> )	22.6	23.6	27.4	25.0	26.2
HbA <sub>1c</sub> (%)	4.1	4.2	5.0	4.6	5.1
75 g OGTT: BS (mg/dl)/IRI (μU/ml)					
0 (min)	76/5.8	–	109/8	81/26	105/7
30	122/29.6	–	188/31	200/353	193/15
60	95/29.0	–	248/45	235/809	224/36
120	94/26.4	–	278/83	93/198	143/65
180	–	–	–	–	80/15
210	–	–	–	–	53/8
240	–	–	–	–	63/10
U-CPR (μg/day)	106.8	107.8	96.1	446.4	205.4
GIR (mg/kg/min)	7.36	3.31	4.17	2.63	5.35
Ultrasound	negative	negative	–	negative	negative
CT	negative	negative	–	negative	negative
MRI	negative	negative	negative	negative	–
Documentation of hypoglycemia	42	56	–	36	40

GIR, glucose infusion rate; –, not examined.

**Table 2.** Clinical features of the normal glucose tolerant and glucose intolerant subjects

	NGT (13)	IGT (12)	Obese NIDDM (31)
M:F	9:4	9:3	21:10
Age (year)	34.9 ± 3.3	47.5 ± 3.5*	44.5 ± 17.9*
BMI (kg/m <sup>2</sup> )	21.1 ± 0.6	21.2 ± 0.8	28.9 ± 3.4**
HbA <sub>1c</sub> (%)	5.3 ± 0.1	5.9 ± 0.3	9.8 ± 2.5**
FPG (mg/dl)	88.8 ± 2.0	97.3 ± 4.2	157.3 ± 48.8**
Fasting IRI (μU/ml)	7.5 ± 0.8	10.3 ± 1.2	18.3 ± 10.6**
GIR (mg/kg/min)	6.31 ± 0.5	6.41 ± 0.7	2.56 ± 1.37**

NGT, normal glucose tolerance; IGT, impaired glucose tolerance; FPG, fasting plasma glucose; GIR, glucose infusion rate; Obese NIDDM, BMI > 27 (male), > 25 (female);

\**P* < 0.05, \*\**P* < 0.001 *vs.* NGT; mean ± SEM.

40 and 42 mg/dl) and a low normal glucose level of 56 mg/dl in one subject during episodes. One was diabetic, one was impaired glucose tolerant, and two were normal glucose tolerant subjects according to the WHO criteria determined by oral glucose challenge test. The remaining subject was not examined. None of the subjects had a history of upper gastrointestinal surgery or endocrine diseases, or had received any medications known to cause hypoglycemia. All the subjects complained of episodic hypoglycemic symptoms such as fatigue, restlessness, palpitation, sweating and tremors 4–5 h after meals. These symptoms were

promptly relieved by eating something sweet. They were carefully examined by ultrasound scanning (US), computed tomography (CT) and magnetic resonance imaging (MRI) without evidence of islet cell tumor in the pancreas. Their symptoms substantially diminished over 12 months in all subjects except one (Case 4), whose hypoglycemia has persisted until the present. For comparison, 13 subjects with normal glucose tolerance (NGT), 12 with impaired glucose tolerance (IGT), and 31 with obese NIDDM (body mass index: >27 kg/m<sup>2</sup> for men; >25 kg/m<sup>2</sup> for women) served as controls (Table 2).

All the subjects gave their permission for participation in clamp studies after detailed explanation of the experimental protocol and possible risks.

#### *Hyperinsulinemic glucose clamp technique*

The suppressibility of plasma C-peptide was examined during the hyperinsulinemic glucose clamp study with an artificial endocrine pancreas (Nikkiso STG-22, Nikkiso Co., Tokyo), as reported previously [6]. Briefly, after an overnight fast, the subjects received a primed-continuous intravenous infusion of insulin (Novolin R, Novo-Nordisk Pharmaceuticals Co., Copenhagen, Denmark) at a rate of 1.12 or 5.0 mU/kg/min. Venous plasma glucose was maintained at around 80 or 60 mg/dl for 60–90 min by infusion of 10% glucose according to the algorithm established by DeFronzo *et al.* [7]. The average glucose infusion rate (GIR) during the final 30 min of steady-state euglycemia in the standard clamp test (peripheral plasma glucose level: 80 mg/dl; insulin infusion rate: 1.12 mU/kg/min) was used as an index of the whole body's insulin sensitivity. In three subjects (Cases 1, 2 and 4), the hyperinsulinemic clamp study was performed twice approximately 12 months apart.

#### *Radioimmunoassay of plasma insulin and C-peptide*

Before insulin infusion and during steady-state glycemia, plasma samples were drawn for determination of insulin (IRI) and C-peptide (CPR). Plasma IRI and CPR concentrations were measured by radioimmunoassay with a Phadeseph Insulin RIA kit (Pharmacia Diagnostics, Sweden) and C-peptide test (Shionogi Research Laboratories, Osaka).

#### *Statistical analysis*

The results are expressed as the means  $\pm$  SEM. Statistical analysis was performed by Student's paired *t*-test.

### Results

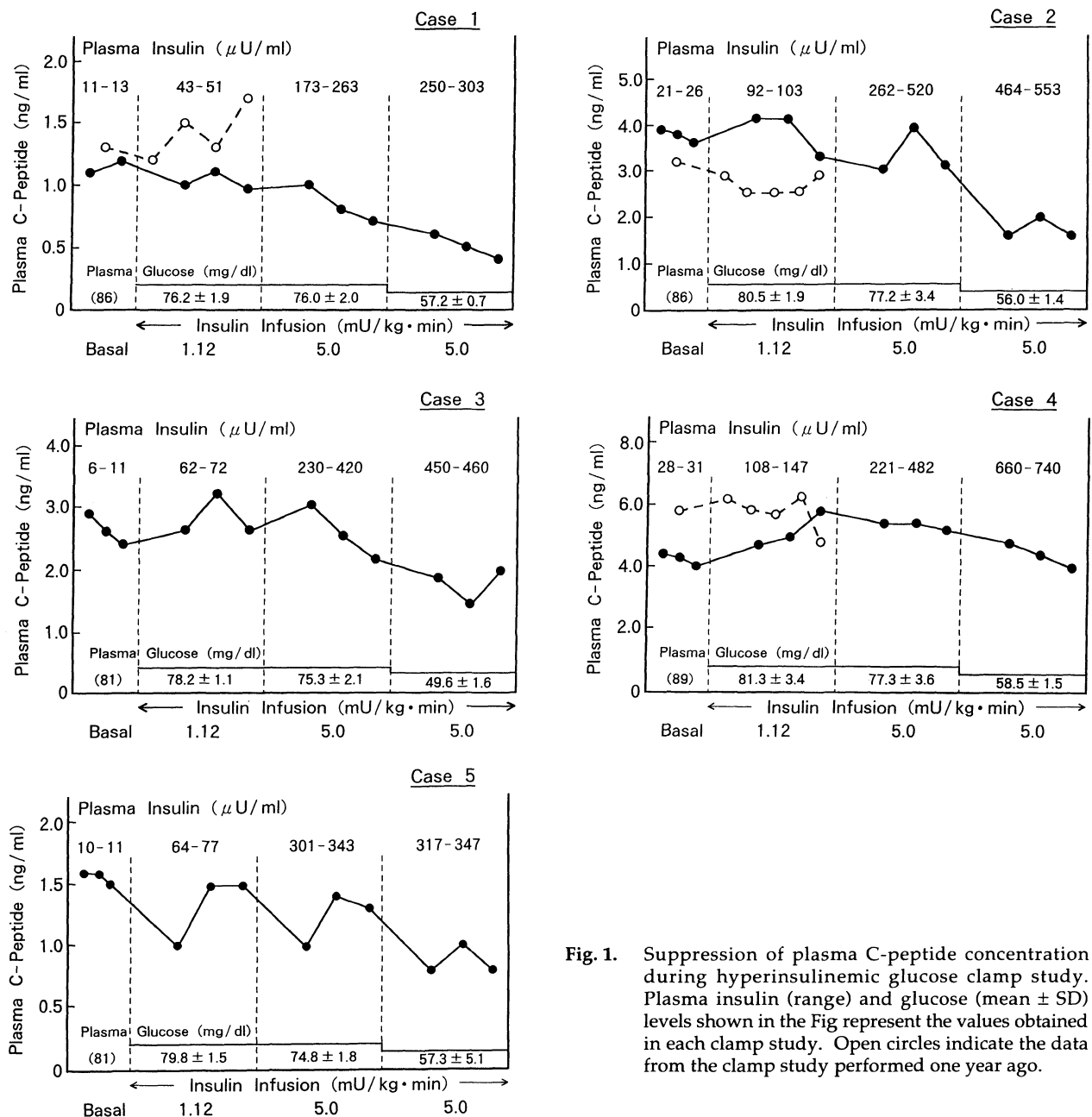
Plasma C-peptide concentrations decreased significantly during the standard euglycemic hyperinsulinemic clamp study (steady-state plasma insulin levels: 50–100  $\mu$ U/ml). The nadir values expressed as % of the basal level were  $37.1 \pm 3.8\%$  (from  $1.64 \pm 0.13$  ng/ml to  $0.61 \pm 0.14$  ng/ml,  $P < 0.001$ ) in NGT,  $46.3 \pm 5.6\%$  (from  $1.93 \pm 0.22$  ng/ml to  $0.89 \pm 0.23$  ng/ml,  $P < 0.001$ ) in IGT, and  $39.9 \pm 2.6\%$  (from  $3.40 \pm 0.74$  ng/ml to  $1.36 \pm 0.31$  ng/ml,  $P < 0.001$ ) in obese NIDDM subjects (Table 3).

In contrast, the five subjects with symptoms suggesting reactive hypoglycemia demonstrated no significant suppression of plasma C-peptide during an identical clamp study (Fig. 1), and similar results were obtained again in each of the three subjects studied after a one year follow-up (Cases 1, 2 and 4). Even supraphysiological hyperinsulinemia (200–500  $\mu$ U/ml) had no effect on the plasma C-peptide level (mean % of basal level:  $90.6 \pm 10.4\%$ , NS), but when the glucose clamp level was lowered to 50–60 mg/dl, the plasma C-peptide level decreased significantly in four of five subjects ( $61.7 \pm 11.5\%$ ,  $P < 0.05$ ). One (Case 4) did not show any reduction in the plasma C-peptide level during supraphysiological hyperinsulinemia either alone or in combination with a low-normal glucose level.

**Table 3.** Maximum suppression of plasma C-peptide (% of baseline)

		Plasma glucose	Insulin infusion rate	Suppression of C-peptide
NGT	(n=13)	80 mg/dl	1.12 mU/kg/min	$37.1 \pm 3.8\%^{**}$
IGT	(n=12)	80	1.12	$46.3 \pm 5.6\%^{**}$
NIDDM	(n=31)	80	1.12	$39.9 \pm 2.6\%^{**}$
Reactive hypoglycemia		80	1.12	$97.3 \pm 8.6$
	(n=5)	80	5.0	$90.6 \pm 10.4$
		60	5.0	$61.7 \pm 11.5^*$

\* $P < 0.05$ , \*\* $P < 0.001$  vs. baseline.



**Fig. 1.** Suppression of plasma C-peptide concentration during hyperinsulinemic glucose clamp study. Plasma insulin (range) and glucose (mean  $\pm$  SD) levels shown in the Fig represent the values obtained in each clamp study. Open circles indicate the data from the clamp study performed one year ago.

## Discussion

All five subjects with symptoms suggesting reactive hypoglycemia in this study showed a lack of suppression of plasma C-peptide during our physiologically and supraphysiologically hyperinsulinemic glucose clamp studies. Suppressibility of C-peptide by exogenous insulin has not been

addressed previously in such subjects.

Many previous studies demonstrated that insulin is able to inhibit its own secretion in normoglycemia in normal [8-11] and NIDDM subjects [12-14]. DeFronzo *et al.* [15] clearly showed with the euglycemic hyperinsulinemic clamp technique that the inhibition of plasma C-peptide concentration by insulin is so sensitive that an increase in plasma insulin by only  $24 \pm 3 \mu\text{U/ml}$

above the baseline level is sufficient to suppress plasma C-peptide secretion in healthy subjects. We previously reported approximately 50% maximum suppression under standard hyperinsulinemic glucose clamp conditions (insulin infusion rate: 1.12 mU/kg/min; venous plasma glucose level: 80 mg/dl) in normal and glucose-intolerant subjects [16], reconfirmed by the present study. The lack of suppression of plasma C-peptide found in the present subjects therefore seems quite unusual.

None of the subjects had ever experienced fasting hypoglycemia, thereby speaking against insulinoma, and one subject (Case 5) studied tolerated up to a 72-h fast without ensuing hypoglycemia. Furthermore, our patients were thoroughly examined for possible occult islet cell tumor in the pancreas by US, CT and MRI, which all gave negative results. The C-peptide suppression test was repeated in three subjects approximately one year later. Unexpectedly, no significant suppression of plasma C-peptide was observed in three individuals, even though they no longer had hypoglycemic episodes. This finding indicates that there is no causal relation between B-cell non-suppressibility and reactive hypoglycemia. On the other hand, plasma C-peptide was suppressed by lowering plasma glucose to the low-normal level (50–60 mg/dl) in four of five subjects. This therefore supports the concept that a glucose-mediated feedback inhibition would be superior to an insulin-mediated one, and seems to provide additional evidence denying the presence of autonomous islet cell tumor in these

subjects, because insulin secretion by insulinoma devoids of suppressibility by hypoglycemia [17, 18]. One subject who was unresponsive to both high levels of insulin and low-normal glucose was unique for his insulin resistance. Further follow-up is clearly necessary for this case.

Concerning the mechanism of non-suppressibility of B-cell function by exogenous insulin in these subjects, it is quite intriguing to note that unexpected hypoglycemia occurs in some recipients of pancreas transplanted allografts [19, 20]. Luzi *et al.* [21] and Boden *et al.* [22] demonstrated the lack of feedback inhibition of insulin secretion by insulin itself in the denervated pancreas, suggesting a neurally mediated mechanism. We have also reported the incomplete suppression of plasma C-peptide by physiological hyperinsulinemia from the transplanted pancreas allograft [23]. *In vitro* studies also demonstrated the lack of direct inhibition of insulin secretion by insulin [24, 25]. From these observations, it seems reasonable to speculate that insulin acts indirectly by neurohumoral activation rather than directly via insulin receptors on B-cells as is generally believed. It is noteworthy that insulin and its receptors are present in the central nervous system [26], and thus inhibition of C-peptide secretion by exogenous insulin could be in part due to the activation of a neuroendocrine control loop [27]. Our study extended this hypothesis by demonstrating similar abnormalities in an apparently functional disorder.

Further studies are needed to elucidate the underlying mechanism in this interesting study.

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