

Insulin deficiency with and without glucagon: A comparative study between total pancreatectomy and type 1 diabetes

Fumimaru Niwano¹, Yoshihisa Hiromine¹ , Shinsuke Noso¹ , Naru Babaya¹ , Hiroyuki Ito¹ , Sara Yasutake¹, Ippei Matsumoto², Yoshifumi Takeyama², Yumiko Kawabata¹, Hiroshi Ikegami^{1*}

Departments of ¹Endocrinology, Metabolism and Diabetes, and ²Surgery, Kindai University Faculty of Medicine, Osaka, Japan

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*Correspondence

Hiroshi Ikegami
Tel.: +81-72-366-0221 ext. 3124
Fax: +81-72-366-2095
E-mail address:
ikegami@med.kindai.ac.jp

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ABSTRACT

Aims/Introduction: Patients with a total pancreatectomy and type 1 diabetes are similar in regard to absolute insulin deficiency, but different in regard to glucagon, providing a unique opportunity to study the contribution of glucagon to glucose metabolism in an insulin-dependent state. The aim of the present study was to investigate the contribution of glucagon to glucose homeostasis in complete insulin deficiency *in vivo*.

Methods: A total of 38 individuals with a complete lack of endogenous insulin (fasting C-peptide <0.0066 nmol/L) and whose glycemic control was optimized with an insulin pump during hospitalization were retrospectively studied. The basal insulin requirement, time-to-time adjustment of the basal insulin infusion rate, prandial insulin requirement and fasting plasma glucagon were compared between patients with a total pancreatectomy ($n = 10$) and those with type 1 diabetes ($n = 28$) after achievement of optimal glycemic control.

Results: Total daily insulin ($P = 0.03$) and basal insulin ($P = 0.000006$), but not prandial insulin requirements, were significantly lower in total pancreatectomy patients than in type 1 diabetes patients. The basal percentage (basal insulin/total daily insulin) was also significantly lower in total pancreatectomy patients than in type 1 diabetes patients (15.8 ± 7.8 vs $32.9 \pm 10.1\%$, $P = 0.00003$). An increase in the insulin infusion rate early in the morning was not necessary in most patients with a pancreatectomy. The fasting plasma glucagon concentration was significantly lower in total pancreatectomy patients than in type 1 diabetes patients ($P = 0.00007$), and was positively correlated with the basal insulin requirement ($P = 0.038$).

Conclusions: The difference in insulin requirements between total pancreatectomy and type 1 diabetes patients suggests a contribution of glucagon to the basal insulin requirement and dawn phenomenon.

INTRODUCTION

Both insulin and glucagon play a central role in glucose homeostasis^{1–4}. Recent studies have suggested an important role of glucagon in glucose homeostasis during insulin deficiency, as suggested by the lack of hyperglycemia and ketoacidosis induced by insulin deficiency in mice deficient for glucagon signaling^{4–6}. Whether this situation occurs in humans remains to be definitively determined.

Total pancreatectomy is unique in that the patient is completely deprived of both insulin and glucagon, as well as other pancreatic hormones. Type 1 diabetes mellitus is caused by a selective destruction of the insulin-producing β -cells of the pancreas, and patients are almost completely deprived of insulin. Patients with type 1 diabetes and those with a total pancreatectomy are similar in terms of their absolute insulin deficiency, but differ in their expression of glucagon and other islet hormones, which are present in type 1 diabetes and completely lacking in a total pancreatectomy. The investigation of the insulin requirements and metabolic profiles in patients with a total

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pancreatectomy is therefore important, not only because of the clinical relevance for controlling diabetes and normalizing energy metabolism in such patients, but also because it provides a unique opportunity to investigate the effect of glucagon deficiency on the insulin requirement and metabolic profile in absolute insulin deficiency *in vivo*⁷. We therefore studied the difference in the insulin requirement and metabolic profiles between patients with type 1 diabetes and those with a total pancreatectomy. To evaluate the insulin requirement as precisely as possible, all patients were treated with an insulin pump, and basal insulin was adjusted on a time-to-time basis in a tightly controlled manner. The results showed a marked decrease in the basal, but not prandial, insulin requirement in patients with a total pancreatectomy compared with patients with type 1 diabetes, suggesting a contribution of glucagon in the maintenance of basal glucose homeostasis.

METHODS

A total of 48 individuals with insulin dependency whose glycemic control was optimized with an insulin pump during hospitalization according to the standard protocol of our department were retrospectively studied. Insulin dependency was defined by fasting C-peptide levels <0.2 nmol/L (0.6 ng/mL)⁸. Among the 48 patients, 10 patients were insulin-dependent, owing to a total pancreatectomy, and 38 were type 1 diabetes patients. Endogenous insulin was completely abolished (fasting C-peptide level <0.0066 nmol/L) in all patients with a total pancreatectomy and in 28 patients with type 1 diabetes, whereas 10 patients with type 1 diabetes showed minimal residual insulin secretion (fasting C-peptide level 0.0066–0.2 nmol/L). Because recent studies have shown that the metabolic manifestations of glucagon deficiency are significantly affected by residual β -cell function¹, data from the 38 patients with a complete lack of endogenous insulin (10 patients with a total pancreatectomy and 28 patients with type 1 diabetes) were used for the analysis. All participants were Japanese and had been treated with intensive insulin therapy with either an insulin pump or with multiple insulin injection therapy. All 10 patients with a total pancreatectomy received pancrelipase and were told to consume 600 mg orally with each primary meal (three meals per day). Patients with type 1 diabetes included 20 patients with acute-onset type 1 diabetes, four patients with slowly progressive type 1 diabetes and four patients with idiopathic type 1 diabetes, including three patients with fulminant type 1 diabetes. Glutamate decarboxylase antibody testing was positive in 10 patients with acute-onset type 1 diabetes, in all four patients with slowly progressive type 1 diabetes and in no patients with idiopathic type 1 diabetes. Patients were excluded if they had eating disorders, concomitant dietary restrictions, unstable retinopathy or renal failure, were pregnant, or were being treated with other antidiabetic agents or steroids. This study was approved by the institutional ethics committee of Kindai University Faculty of Medicine. The

informed consent requirement was waived because this research used a retrospective study design.

All 38 participants were hospitalized to achieve optimal glycemic control with insulin pump therapy using Paradigm 712, 722 or Minimed 620G pumps (Medtronic, Northridge, CA, USA). A rapid-acting insulin analog was used in all patients. To exclude factors that could modify insulin dose requirements, all patients were provided meals consisting of a constant nutrient balance prepared by dietitians. The prescribed meals were based on standard meals for diabetes patients recommended by the Japan Diabetes Society; the total energy was 25–30 kcal/kg ideal bodyweight, consisting of 50–60% carbohydrate, 15–20% protein and 20–25% fat.

Optimization of the basal insulin infusion rates was made so that the blood glucose levels remained steady (change in glucose level <1.67 mmol/L) during the fasting procedure. The fasting procedure was carried out by skipping a meal in the morning (skipping breakfast), afternoon (skipping lunch) or evening and overnight (skipping dinner), and monitoring the glycemic excursion thereafter until the next meal. A nine-point glucose test (before and 2 h after each meal, before bedtime, at midnight [0:00] and 3:00 am) was carried out to allow the optimization of the basal insulin rates.

The prandial insulin dose was determined as follows. All meals were consumed within 20 min, and no additional food or drink was consumed unless required to treat hypoglycemia. The target glucose level was set from 5.6 to 7.2 mmol/L before each meal and below 10.0 mmol/L 2 h after each meal, and the prandial insulin rates were optimized to achieve this target.

The morbus value (M-value)⁹ was calculated to assess glycemic excursion of daily profiles of blood glucose levels. The M-value is a logarithmic transformation of the deviation of glycemic levels from an arbitrarily assigned 'ideal' glucose value, which shows both the mean glucose value and the effect of glucose swings. Each individual M-value for each blood glucose value was calculated as: (absolute value of $[10 \times \log(\text{blood glucose value}/120)]^3$).

Fasting blood samples to measure the C-peptide, glycated hemoglobin, albumin, total cholesterol, glucagon, growth hormone, free thyroxine, cortisol and anti-insulin antibody levels were obtained in the morning after an overnight fast.

Laboratory measurements

The C-peptide concentration was measured through a chemiluminescent enzyme immunoassay (Lumipulse Presto[®] C-peptide commercial kit; Fujirebio Co., Tokyo, Japan). The detection limit of the assay was 0.0066 nmol/L (0.02 ng/mL).

Anti-insulin antibody levels were measured by radioimmunoassay (Insulin Ab RIA Assay kit; RSR Ltd., Cardiff, UK). The measuring range was 0.4–50 U/mL. Anti-insulin antibody levels lower than the sensitivity of the measurement (0.4 U/mL) were calculated as 0 U/mL, and those higher than the upper limit of the measurement (50 U/mL) were calculated as 50 U/mL.

The plasma glucagon concentration was measured with a sandwich enzyme-linked immunosorbent assay using N- and C-terminal-specific glucagon antibodies (Mercodia Glucagon ELISA; Mercodia, Uppsala, Sweden). The detection limit of the assay was 6.1 pg/mL. Plasma glucagon levels lower than the sensitivity of the measurement (6.1 pg/mL) were calculated as 0 pg/mL.

Statistical analysis

All data are expressed as the mean \pm standard deviation. Categorical variables are expressed numerically as percentages. Statistical analyses were carried out using Bell Curve for Excel software (Social Survey Research Information Co., Ltd., Tokyo, Japan). We used Student's *t*-tests to analyze continuous variables and χ^2 -tests for categorical variables. Statistical significance was defined as $P < 0.05$.

RESULTS

The clinical characteristics of the participants are shown in Table 1. Patients with a total pancreatectomy were older, had a longer duration of diabetes, lower bodyweight, lower body mass index and lower glycated hemoglobin than patients with type 1 diabetes. The fasting C-peptide levels were below the limit of detection (0.0066 nmol/L) in all patients. There was no significant difference in nutritional parameters, such as serum albumin and total cholesterol levels, between patients with a total pancreatectomy and patients with type 1 diabetes.

The blood glucose profiles after optimization of basal and bolus insulin administration were comparable between patients with a total pancreatectomy and type 1 diabetes patients at 7 points during the day (Figure S1) and at 3 points overnight (Figure S2), except for a slightly higher glucose level in patients with a total pancreatectomy before lunch. The M-value of the 7 points of daily profiles of blood glucose was not significantly different between patients with a total pancreatectomy and patients with type 1 diabetes (11.7 ± 7.3 vs 8.8 ± 4.8 , not significant). The insulin requirements after optimization of glycaemic control are shown in Table 2 and Figure 1. The total daily

dose of insulin in patients with total pancreatectomy (21.9 ± 6.0 units/day) was significantly lower than that in type 1 diabetes (35.9 ± 13.3 units/day, $P = 0.003$). The basal insulin dose was significantly lower in the total pancreatectomy group (3.7 ± 2.4 units/day) than in the type 1 diabetes group (11.4 ± 4.6 units/day, $P = 0.00001$). The proportion of the basal insulin dose to the total daily dose (basal percentage) was also significantly lower in the total pancreatectomy group than in the type 1 diabetes group (15.8 ± 7.8 vs $32.9 \pm 10.1\%$, $P = 0.00003$). The prandial insulin dose in the total pancreatectomy group (18.2 ± 4.4 units/day) was not significantly different from that in the type 1 diabetes group (24.6 ± 11.1 units/day, not significant; Table 2).

For normalization to the difference in bodyweight, which was significantly lower in those with a total pancreatectomy than in those with type 1 diabetes, the insulin requirement was expressed by units per kg bodyweight. The total daily insulin (0.494 ± 0.104 vs 0.666 ± 0.232 units/kg, $P = 0.03$) and basal insulin dose (0.081 ± 0.047 vs 0.210 ± 0.072 units/kg, $P = 0.000006$), but not the prandial insulin dose (0.414 ± 0.085 vs 0.458 ± 0.211 , not significant), were significantly lower in patients with a total pancreatectomy than in those with type 1 diabetes, respectively (Table 2).

To compare the pattern of insulin infusion on a time-to-time basis, the rate of insulin infusion per hour was examined (Figure 1). The insulin infusion rate in the total pancreatectomy group was significantly lower than in the type 1 diabetes group at all time-points. An increase in the insulin infusion rate early in the morning (the dawn phenomenon) was observed in patients with type 1 diabetes (Figure 1), but this was not observed in those with a total pancreatectomy. The difference between the maximum and the minimum infusion rate during the night (from 0:00 to 6:00) in the total pancreatectomy group was significantly smaller than in the type 1 diabetes group (0.03 ± 0.04 vs 0.17 ± 0.13 units/h, $P = 0.001$). The difference between the maximum and the minimum infusion rate at dusk (from 15:00 to 21:00) in patients with total pancreatectomy was comparable with that in the type 1 diabetes patients (0.03 ± 0.04 vs 0.07 ± 0.10 units/h, not significant).

Table 1 | Clinical characteristics of total pancreatectomy patients and type 1 diabetes patients

	Total pancreatectomy	Type 1 diabetes	<i>P</i> -value
Sex (male/female)	4/6	7/21	NS
Age (years)	69.0 ± 16.6	47.6 ± 16.5	0.001
Diabetes duration (years)	3.3 ± 4.9	12.0 ± 8.8	0.006
Height (m)	1.54 ± 0.10	1.58 ± 0.09	NS
Weight (kg)	44.0 ± 6.2	53.8 ± 9.9	0.006
BMI (kg/m^2)	18.5 ± 2.6	21.3 ± 2.9	0.01
HbA1c (%)	7.34 ± 1.36	8.55 ± 1.40	0.02
Total cholesterol (mmol/L)	4.51 ± 0.66	4.95 ± 0.71	NS
Serum albumin (g/L)	37.4 ± 4.7	40.2 ± 4.2	NS

Data are mean \pm standard deviation. HbA1c, glycaemic hemoglobin; BMI, body mass index; NS, not significant.

Table 2 | Insulin requirements in total pancreatectomy patients and type 1 diabetes patients after optimization of basal and prandial insulin with an insulin pump (continuous subcutaneous insulin infusion)

		Total pancreatectomy (n = 10)	Type 1 diabetes (n = 28)	P-value
Total daily insulin	Unit/day	21.9 ± 6.0	35.9 ± 13.3	0.003
	Unit/kg/day	0.494 ± 0.104	0.666 ± 0.232	0.03
Basal insulin	Unit/day	3.7 ± 2.4	11.4 ± 4.6	0.00001
	Unit/kg/day	0.081 ± 0.047	0.210 ± 0.072	0.000006
Prandial insulin	Unit/day	18.2 ± 4.4	24.6 ± 11.1	NS
	Unit/kg/day	0.414 ± 0.085	0.458 ± 0.211	NS
Basal percentage [†]	%	15.8 ± 7.8	32.9 ± 10.1	0.00003

Data are mean ± standard deviation. [†]Basal insulin dose/total daily dose. NS, not significant.

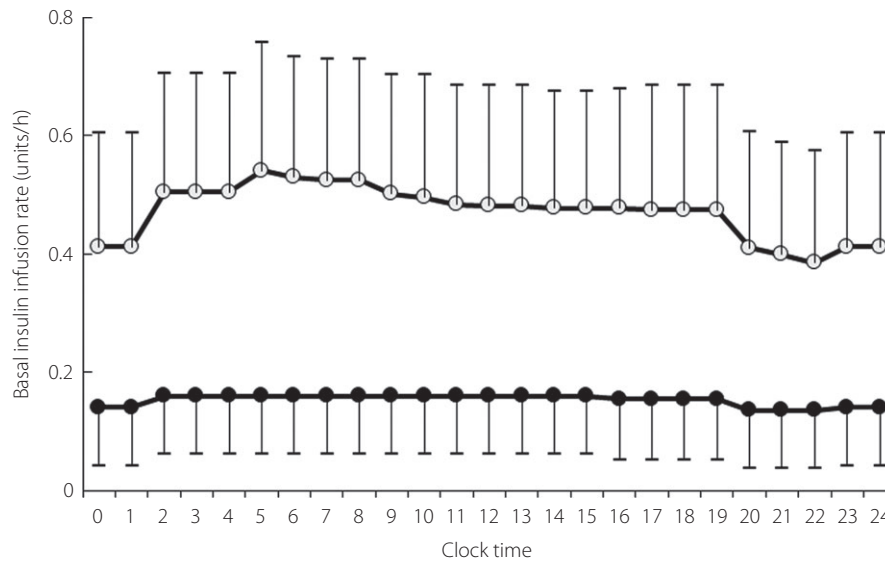


Figure 1 | Basal insulin requirements (units/h) in total pancreatectomy patients (closed circle) and type 1 diabetes patients (open circle), whose glycemic control was optimized by using an insulin pump. Data are expressed as the mean ± standard deviation.

The plasma glucagon concentration was available in a subset of 31 participants (24 type 1 diabetes patients and 7 total pancreatectomy patients). The plasma glucagon concentration in the total pancreatectomy group was significantly lower than in the type 1 diabetes group (6.3 ± 6.9 vs 28.7 ± 12.2 pg/mL, $P = 0.00007$; Figure 2a). The basal insulin dose was positively correlated with the fasting plasma glucagon concentration when considering both types of diabetes in combination ($R = 0.38$, $P = 0.038$; Figure 2b).

There was no significant correlation between the fasting plasma glucagon concentration and the fasting glucose concentration, a potential confounding factor for glucagon levels (Figure S3). There was no difference in growth hormone, free thyroxine and cortisol levels between patients with a total pancreatectomy and type 1 diabetes patients (Table S1). There was no difference in anti-insulin antibody levels between patients with a total pancreatectomy and type 1 diabetes patients (10.1 ± 12.2 vs 11.2 ± 19.8 U/mL, not significant).

DISCUSSION

The present study showed that the characteristics of diabetes mellitus secondary to total pancreatectomy differed greatly from patients with type 1 diabetes. A difference was found in age, bodyweight, body mass index and age of onset, with older age, leaner bodyweight and older age of onset of diabetes associated with a total pancreatectomy. These results might reflect differences in the characteristics of the original diseases, namely pancreatic diseases and type 1 diabetes.

Of note is the marked difference in the insulin requirements between the two groups. The total daily insulin requirement and basal insulin dose, but not the prandial insulin dose, were markedly and significantly smaller in patients with a total pancreatectomy than in those with type 1 diabetes. In particular, the basal insulin dose in total pancreatectomy was as low as 3.7 units/day, which was less than one-third the dose required in type 1 diabetes. The basal insulin as a percentage of total daily insulin was also markedly and significantly smaller in the

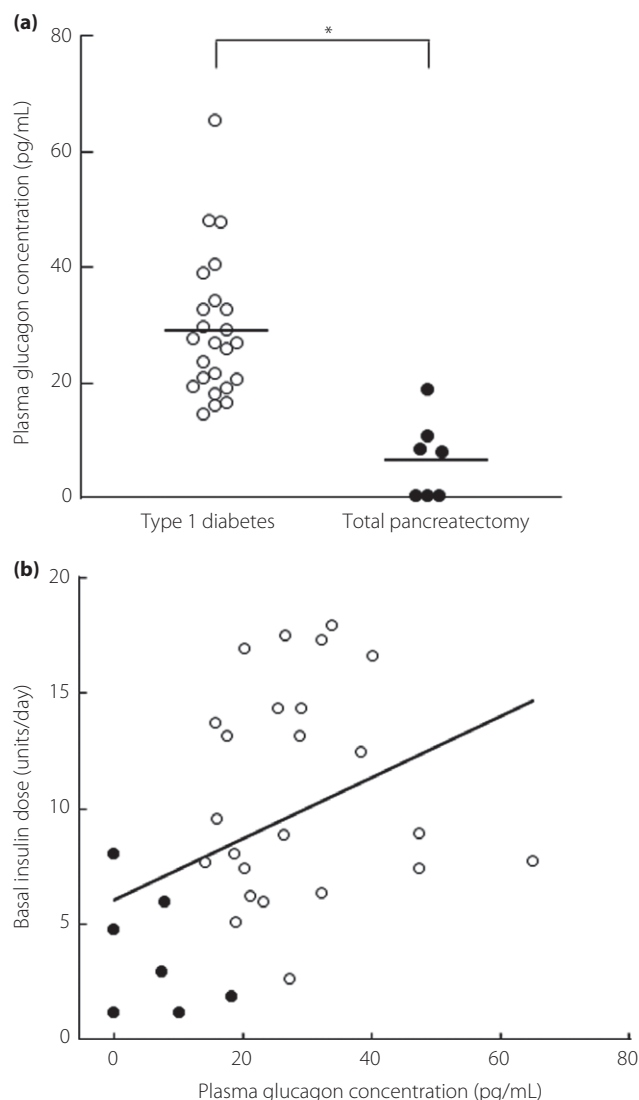


Figure 2 | (a) Plasma glucagon concentration (pg/mL) in total pancreatectomy patients (closed circle) and type 1 diabetes patients (open circle). All individual data-points and means are shown. The plasma glucagon concentration in the total pancreatectomy group was significantly lower than that in the type 1 diabetes group (6.3 ± 6.9 vs 28.7 ± 12.2 pg/mL, $P = 0.00007$). * $P = 0.00007$ vs type 1 diabetes. (b) Correlation between the fasting plasma glucagon concentration and basal insulin dose in total pancreatectomy patients (closed circle) and type 1 diabetes patients (open circle). The basal insulin requirement was positively associated with the fasting plasma glucagon concentration ($R = 0.38$, $P = 0.038$). However, the correlation was not statistically significant within each group (total pancreatectomy: $R = 0.43$, not significant; type 1 diabetes: $R = 0.02$, not significant).

total pancreatectomy group than the type 1 diabetes group (15.8% vs 32.9%).

The two groups were identical in terms of the endogenous insulin secretory capacity, with no detectable C-peptide in either group. The glucose levels were also similarly optimized by use of insulin pumps, and other environmental factors were

also normalized by hospitalization and by providing a standard diet in both groups. The difference in bodyweight, which was significantly lower in the total pancreatectomy group than the type 1 diabetes group, was also not the main reason for the difference in insulin requirements, because it was observed even after normalization of bodyweight. Another possible explanation for the difference is a difference in the digestion and absorption of nutrients, which might be incomplete in patients with a total pancreatectomy. Patients with a total pancreatectomy, however, received supplementation with pancreatic enzyme medicine after a total pancreatectomy, no diarrhea or steatorrhea was observed and the nutritional status was comparable with that of type 1 diabetes patients. In addition, the prandial insulin requirement was similar between those with a total pancreatectomy and type 1 diabetes, thus suggesting that the digestion and absorption of nutrients was less likely to be the reason for the difference in the basal insulin requirement between the two groups.

Another possible reason for the marked difference in the basal insulin requirement between patients with a total pancreatectomy and those with type 1 diabetes is glucagon. Whereas type 1 diabetes patients lack insulin secretion, patients with a total pancreatectomy lacked not only insulin, but also glucagon, a well-known counter-regulatory hormone of insulin. In fact, the plasma levels of pancreatic glucagon were significantly lower in patients with a total pancreatectomy than in those with type 1 diabetes (Figure 2a). The levels of other counter-regulatory hormones, such as growth hormone and cortisol, and anti-insulin antibody, which might affect insulin requirements, were comparable between patients with a total pancreatectomy and those with type 1 diabetes. The positive correlation between the plasma level of glucagon and the basal insulin requirement further supports this hypothesis (Figure 2b). The correlation, however, was not statistically significant when the patients with a total pancreatectomy and those with type 1 diabetes (Figure 2b, closed circles and open circles, respectively) were analyzed separately. In addition, enhanced glucagon responsiveness was previously reported in patients with a pancreatectomy¹⁰. Further studies with a larger number of participants are necessary to draw definitive conclusions regarding the contribution of glucagon to basal insulin requirements.

A time-to-time adjustment of the basal insulin infusion rate is required to optimize glycemic control in patients with no residual β -cell function. In particular, an increase in the insulin infusion rate early in the morning is often necessary to overcome the so-called 'dawn phenomenon' in patients with type 1 diabetes with no residual β -cell function. Patients with a total pancreatectomy required a much smaller amount of basal insulin than those with type 1 diabetes at any time-point throughout the day (Figure 1). In addition, an increase in the insulin infusion rate was not necessary in most patients, thus showing that the dawn phenomenon was less pronounced in total pancreatectomy patients than in type 1 diabetes patients.

Because the dawn phenomenon is often difficult to manage clinically, even when using a long-acting insulin analog, and the only possible solution currently available is a pre-programmable insulin pump, a method to overcome the dawn phenomenon in the normal clinical setting has long been awaited. The data in the present study suggest that glucagon antagonism might be a solution to overcome the dawn phenomenon without using an insulin pump. Because glucagon antagonism has been proposed as a potential therapeutic target for type 2 diabetes¹¹, and a number of glucagon receptor antagonists are either under clinical trial or in the preclinical phase^{11–13}, studying whether the stabilization of glycemic control can be achieved with glucagon antagonism in type 1 diabetes patients with a complete lack of insulin is necessary.

In contrast to the complete lack of endogenous insulin, as assessed on the basis of a plasma C-peptide level below the detection limit, pancreatic glucagon was detectable, albeit at very low levels, in four out of seven patients with a total pancreatectomy. Cross-reactivity of glucagon-related peptides, such as oxyntomodulin and glicentin, in the glucagon assay has long been under debate, but sandwich enzyme-linked immunosorbent assays using a combination of C- and N-terminal anti-glucagon antibodies, as used in the present study, have been reported to detect pancreatic glucagon with very small cross-reactivity with other glucagon-related peptides^{14,15}. Previous studies have suggested that pancreatic glucagon is synthesized and secreted from the gut^{16–18} at levels detectable even in patients with total pancreatectomy¹⁸, and that such gut-derived glucagon is secreted in response to glucose intake and significantly affects glucose homeostasis¹⁸. The pancreatic glucagon detected in the present study might be derived from the gut. Although detectable, the amount of glucagon was markedly and significantly lower than that in the type 1 diabetes patients, thus potentially explaining the differences in insulin requirements between patients with a total pancreatectomy and those with type 1 diabetes.

Difficulty in glycemic control in patients with a total pancreatectomy is often experienced in clinical practice, likely due to a lack of both insulin and glucagon. In the present study, however, glycemic control in patients with a total pancreatectomy was optimized to levels comparable with those observed in patients with type 1 diabetes. This is likely because all patients were treated with insulin pumps, making it possible to adjust basal insulin infusion rates even at very low doses, as is often necessary for patients with a total pancreatectomy.

There were several limitations to the present study. First, the difference in age between the two groups might have affected the insulin requirements and the extent of the dawn phenomenon. Second, the number of participants was relatively small. Third, catecholamine levels, whose response to insulin-induced hypoglycemia are reportedly reduced in patients with total pancreatectomy¹⁹, were not measured. Further studies are necessary to determine definitive conclusions regarding the

contribution of glucagon to basal insulin requirements and the dawn phenomenon.

In conclusion, the present study showed that the basal insulin requirement in total pancreatectomy patients was considerably different from that in type 1 diabetes mellitus patients, possibly because of differences in glucagon secretion. Such differences should be considered in treating patients with total pancreatectomy. The present study also suggests the possibility of using anti-glucagon therapy for the stabilization of glycemic control in patients with insulin deficiency.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

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

Figure S1 | Seven-point blood glucose profile at the optimization of the basal insulin infusion rates of insulin pump in patients with total pancreatectomy (closed circle) and type 1 diabetes (open circle) during the day.

Figure S2 | Three-point blood glucose profiles for the optimization of the basal insulin infusion rates of insulin pump in patients with total pancreatectomy (closed circle) and type 1 diabetes (open circle) during the night.

Figure S3 | Correlation between the fasting glucose concentration and the fasting plasma glucagon concentration in patients with total pancreatectomy (closed circle) and type 1 diabetes (open circle).

Table S1 | Plasma concentrations of counter-regulatory hormones in patients with total pancreatectomy and type 1 diabetes