Comparison of the efficacy and safety of insulin degludec/aspart (twice-daily injections), insulin glargine 300 U/mL, and insulin glulisine (basal–bolus therapy)

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ABSTRACT
Aims/Introduction: We compared the efficacy and safety of insulin degludec/aspart (IDegAsp) twice-daily injections with insulin glargine 300 U/mL and insulin glulisine basal–bolus therapy (Gla300/Glu) using insulin glargine 300 U/mL (Gla300) and insulin glulisine (Glu).

Materials and Methods: A total of 20 patients with type 2 diabetes mellitus were treated with IDegAsp twice-daily injections; achievement of target preprandial glucose concentration of 100–130 mg/dL at breakfast and supper was determined using a wearable flash glucose monitoring system. Patients were later switched to Gla300/Glu basal–bolus therapy before breakfast and before supper. Data were collected on days 2–4 and days 12–14 for each treatment period. The study's primary efficacy end-point was the mean percentage of time with a target glucose range of 70–180 mg/dL, and safety end-points were the mean percentage of time with hypoglycemia having glucose levels <70 mg/dL, clinically important hypoglycemia with glucose levels <54 mg/dL and nocturnal (00.00–06.00) hypoglycemia.

Results: Considering efficacy, the mean percentage of time for the target glucose range of IDegAsp was significantly lower than that of Gla300/Glu (73.1 [69.4–81.1] vs 84.2 [80.2–93.1], \( P = 0.001 \)). Considering safety, the mean percentages of hypoglycemia (<70 mg/dL; 2.1 [0.0–9.4] vs 14.4 [4.4–22.3]), clinically important hypoglycemia (<54 mg/dL; 0.0 [0.0–0.2] vs 1.9 [0.0–5.6]) and nocturnal (00.00–06.00 hours) hypoglycemia (0.5 [0.0–5.9] vs 8.9 [3.1–11.8]) of Gla300/Glu were significantly lower than those of IDegAsp (\( P = 0.012, 0.036 \) and 0.007, respectively).

Conclusions: When compared with the IDegAsp twice-daily injections, Gla300/Glu basal–bolus therapy might achieve more effective glycemic control without hypoglycemic risk.

INTRODUCTION
Type 2 diabetes mellitus is a progressive disease characterized by the coexistence of insulin action and insulin resistance, and accompanied by hyperglycemic microvascular complications and macrovascular complications. Basal insulin treatment can be used to prevent these complications; however, changing or adding insulin is necessary when the therapeutic goal is unmet.

It is considered that adding bolus insulin to basal insulin or changing to a biphasic insulin preparation strengthens treatment, but it has been reported that this change increases the risk of hypoglycemia and bodyweight gain, as compared with basal supported oral therapy. Thus, strengthening insulin therapy increases the risk of hypoglycemia, and severe hypoglycemia increases cardiovascular events and total mortality. It has also been reported that nocturnal unawareness of hypoglycemia occurs at a higher frequency than what is thought, including in patients with favorable glycated hemoglobin...
(HbA1c) by insulin treatment. Therefore, it is imperative that treatment options for diabetes achieve good glycemic control while avoiding hypoglycemia. It has been reported that in daily practice, doctors do not change the treatment when the therapeutic goal of diabetes patients has not been achieved; however, intervention at an appropriate time is necessary for improved results.

Recommended algorithms for antihyperglycemic therapy for patients with type 2 diabetes mellitus are described in the Standards of Medical Care in Diabetes 2018. If the HbA1c level is not properly controlled, patients treated with basal insulin are recommended for combination injectable therapy. For this therapy, treatments including additional bolus insulin once before the largest meal (basal–bolus), the addition of glucagon-like peptide-1 receptor agonist or changing to premixed insulin twice-daily are recommended as the next steps. Recently approved insulin degludec/aspart (IDegAsp) has achieved a reduction in total hypoglycemia and nocturnal hypoglycemia, as well as good glycemic control when compared with the conventionally used biphasic insulin aspart 30 (BIAsp30) twice-daily injections.

There were a few studies suggesting that HbA1c was not reduced using the conventional drug when compared with basal–bolus insulin treatment. Although basal–bolus reduced HbA1c better than premix insulin twice-daily injections, this treatment becomes complex, as it is now necessary to regulate the glycemic control of two insulin products using different titration methods. No reports have directly compared and examined IDegAsp twice-daily injection and basal–bolus insulin glargine 300 U/mL and insulin glulisine (Gla300/Glu).

In the present study, IDegAsp and Gla300/Glu were compared for their efficacy on glycemic control and their safety. Patients with type 2 diabetes mellitus were hospitalized, and their dietary intake and exercise were monitored while receiving the two insulin therapies.

**METHODS**

**Study design and participants**

The present single-center, open-labeled, single-arm, two-period study of patients with type 2 diabetes mellitus was carried out from January to May 2018. The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013, and this study protocol was approved by the local ethics committee of Minami Osaka Hospital (No. 2017-11). The clinical trial registration number is UMIN 000030648 (University Hospital Medical Information Network Clinical Trial Registry). All participants gave written informed consent after explanation of the study objectives.

A total of 20 participants with type 2 diabetes mellitus, including eight men and 12 women, were registered for participation in a glycemic control and diabetes educational study at the Minami Osaka Hospital, Osaka, Japan. The criteria for selection were as follows: age ≥20 years, diagnosed with type 2 diabetes mellitus on the basis of American Diabetes Association Criteria for >1 year before the present study, having 70% insulin degludec (IDeg) and 30% insulin aspart (IASp), IDegAsp (Ryzodeg; Novo Nordisk A/S, Bagsvaerd, Denmark) twice-daily injections therapy for at least 6 months before the present study and/or having oral hypoglycemic agents (OADs), and glycated hemoglobin (HbA1c) levels >7.0% (52 mmol/mol) and <10.5% (90 mmol/mol), respectively, at screening. Patients with untreated retinopathy, diabetic kidney disease (moderate-to-severe hypofiltration phase) with a moderately decreased estimated glomerular filtration rate (eGFR; <45 mL/min/1.73 m²), severe diabetic neuropathy or nephrotic proteinuria, pregnant women, history of digestive tract surgery, past history of a malignant tumor, severe heart failure of the class IV category of severity classification of heart failure (New York Heart Association), acute coronary syndrome within 12 weeks of the start of this study, and cases of liver failure and liver cirrhosis were excluded from the present study.

The study protocol is shown in Figure 1. All participants received IDegAsp twice-daily injections before hospitalization. After hospitalization, IDegAsp dosage was titrated to a target prandial glucose concentration of 100–130 mg/dL at breakfast and supper on the basis of self-monitoring of blood glucose in the titration period 1 (Figure 1). The titration period after hospitalization lasted over a week to eliminate the influence of glucose toxicity, and the IDegAsp dosage was often titrated over no more than 3 days. After having fixed an insulin dosage, we confirmed the absence of hypoglycemia (<70 mg/dL) by self-monitoring of blood glucose and hypoglycemic symptoms. We then evaluated glycemic control using the Freestyle Libre Pro flash glucose monitoring (FGM) system (Abbott Diabetes Care, Alameda, CA, USA) worn by participants for 15 days. When using the FGM system, the interstitial fluid glucose fluctuation is known to be large on day 1 of the 15-day measurement period, with stable data from the day 2 to day 14. The 15th day, the desorption day, was excluded from the object of evaluation. Therefore, evaluation of the FGM system of IDegAsp was carried out from the days 2 to 4, whereas FGM of Gla300/Glu (Sanofi, Paris, France) was carried out from days 12 to 14. The participants did not change OADs for the study period. The sulfonylurea and glinide agents that caused insulin secretion independent of blood glucose were discontinued >1 week before wearing the FGM. The change from IDegAsp to Gla300/Glu occurred on the 5th day after the FGM was worn. On days 5 and 6, 80% of the dosage of basal insulin IDeg contained in IDegAsp was switched to Gla300 before breakfast, as the half-life of IDeg is as long as 25.4 h. The same dosage of Asp contained in IDegAsp was switched to Glu and injected before supper, the largest meal in titration period 2 (Figure 1). After day 7, Gla300 was injected before breakfast and at the same dosage of basal insulin IDeg contained in IDegAsp. Glu was titrated to postprandial glucose levels, 130–150 mg/dL, 2 h after supper in titration period 3 (Figure 1). Serum albumin was measured, as it is considered to influence the effect of
IDegAsp from diurnal variation during the day, with a minimum of 06.00 and a maximum of 21.00. Each participant was given the same calorie and carbohydrate quantity of the following hospital diet: approximately 28 kcal per kg of ideal bodyweight a day with following the calorie ratio: carbohydrate 0.6, proteins 0.17, lipids 0.23, breakfast 0.3, lunch 0.35 and supper 0.35. Excessive exercise was prohibited, with patients allowed to do moderate exercises for approximately 30 min per day in the testing period.

Outcome measures

The primary end-points of the present study were dependent on the efficacy and safety data provided by the FGM. The efficacy outcome was the mean percentage of time with a target glucose range of 70–180 mg/dL for each treatment period. The safety outcome, in contrast, was the mean percentage of time with hypoglycemia (glucose levels <70 mg/dL). Clinically important hypoglycemia (glucose levels <54 mg/dL) and nocturnal (00.00–06.00 hours) hypoglycemia (<70 mg/dL) for each treatment period. Secondary end-points included the mean percentage of time with hyperglycemia (glucose levels ≥180 mg/dL), 24-h standard deviation of the glucose levels; 24-h mean value (target glucose levels 100 mg/dL), 24-h coefficients of variation (CV) of the glucose levels; CV of the nocturnal (00.00–06.00 hours) glucose levels; the mean amplitude of glycemic excursion calculated from the FGM data considering the glycemic peaks and nadirs; the mean of daily difference used as an index of day-to-day glucose variability; 24-h mean glucose levels; nocturnal (00.00–06.00 hours), morning (08.00–12.00 hours) and afternoon (12.00–24.00 hours) mean glucose levels; preprandial glucose levels at breakfast, lunch and supper; and postprandial glucose level 2 h after supper.

Statistical analysis

All data collected were presented as the median (interquartile range), and the Shapiro–Wilk test was carried out to determine whether data were normally distributed. Statistical analysis was carried out with Wilcoxon’s signed rank test as the significance test, and Spearman’s rank correlation as the test of correlation coefficient, with a two-tailed P-value <0.05 considered significant. From the post-hoc power analysis, with an α value of 0.05 and a statistical power of 80%, the required number of samples was determined to be 11; therefore, we believe the number of cases in the present study was sufficiently satisfied. The data were analyzed using EZR 1.37 software (Saitama Medical Center, Jichi Medical University, Saitama, Japan).

RESULTS

Participant characteristics

Table 1 shows the participants’ characteristics, and all participants completed the study. The study included eight men and 12 women, 10 of which were treated with insulin only, and others treated with insulin + OADs; no significant difference was found between the two groups. The dosage of IDegAsp when the use of FGM began was 8.0 U (6.0–14.0 U) before...
Table 1 | Clinical characteristics of study participants

|                  | IDegAsp | Gla300/Glu | P-value* |
|------------------|---------|------------|----------|
| Subjects (n)     | 20      | –          |          |
| Male, n (%)      | 8 (40.0)| –          |          |
| Age (years)      | 73.0 (67.3–78.5)| –      |          |
| Duration of diabetes (years) | 14.0 (5.8–17.0) | – |          |
| BMI (kg/m²)      | 25.5 (22.5–26.5) | – |          |
| HbA1C (%)        | 8.7 (7.8–9.1) | – |          |
| S-CPR (ng/mL)    | 2.3 (1.3–4.3) | – |          |
| FPG (mg/dL)      | 162.0 (115.0–197.0) | – |          |
| eGFR (mL/min/1.73 m²) | 1.3 (0.8–2.2) | – |          |
| LDL (mg/dL)      | 146.0 (92.0–217.0) | – |          |
| HDL (mg/dL)      | 74.0 (60.5–110.0) | – |          |
| Complications, no. patients (%) | 42.0 (29.8–60.5) | – |          |
| Retinopathy (SDR) | 55.8 (48.4–69.4) | – |          |
| Nephropathy       | 14 (70.0) | – |          |
| Neuropathy        | 17 (85.0) | – |          |
| Other than insulin | 4 (20.0) | – |          |
| DPP4 inhibitor (n) | 10 | – |          |
| Metformin (n)    | 5 | – |          |
| α-Gl (n)          | 7 | – |          |
| Insulin treatment |        |            |          |
| IDegAsp dosage (U/day) | 16.0 (12.0–22.0) | – |          |
| IDegAsp dosage before breakfast (U) | 8.0 (6.0–14.0) | – |          |
| IDegAsp dosage before supper (U) | 8.0 (6.0–11.0) | – |          |
| Ultra-rapid-acting insulin dosage (U/day) | 5.0 (4.0–6.5) | 4.0 (3.0–4.3) | 0.004* |
| Gla300 dosage (U/day) | 11.0 (8.0–15.5) | – |          |
| Serum albumin (g/dL) | |          |          |
| 06.00 hours       | 3.7 (3.6–3.9) | 3.7 (3.5–3.7) | 0.018* |
| 21.00 hours       | 3.8 (3.7–4.0) | 3.8 (3.7–3.8) | 0.233 |

Values are expressed as median (interquartile). *Data were compared using the Wilcoxon’s signed rank test. A P-value of <0.05 was considered significant. Antidiabetic drugs other than insulin remained the same during the study: α-Gl, alpha-glucosidase inhibitor; BMI, body mass index; CPI, C-peptide index; DPP4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; Gla300, glargine 300 U/mL; Glu, insulin glulisine; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; IDegAsp, insulin degludec/aspart; LDL, low-density lipoprotein; S-CPR, serum C-peptide immunoreactivity; SDR, simple diabetic retinopathy; TG, triglyceride.

Comparison of the efficacy and safety between IDegAsp and Gla300/Glu

The primary end-points of the present study were the mean percentage of time with a target glucose range of 70–180 mg/dL as efficacy, and hypoglycemia with glucose levels of <70 mg/dL as safety for each treatment period. The mean percentage of time within the target glucose range for IDegAsp was significantly lower than Gla300/Glu (P = 0.001), and the mean percentages of hypoglycemia (<70 mg/dL), clinically important hypoglycemia (<54 mg/dL) and nocturnal (00.00–06.00) hypoglycemia of Gla300/Glu were significantly lower than IDegAsp (P = 0.012, 0.036 and 0.007, respectively).

Regarding the secondary end-points, the mean percentage of time with hyperglycemia (≥180 mg/dL); 24-h mean glucose level; the nocturnal (00.00–06.00 hours), morning (08.00–12.00 hours) and afternoon (12.00–24.00 hours) mean glucose levels; preprandial glucose levels at breakfast, lunch and supper; and postprandial glucose levels 2 h after lunch and supper were insignificantly different between the IDegAsp and Gla300/Glu groups. In contrast, the 24-h standard deviation of glucose levels, 24-h mean value, 24-h CV of glucose levels, nocturnal (00.00–06.00 hours) CV and mean amplitude of glycemic excursion (the indicators of diurnal variation of glucose levels), and the mean of daily difference (the index of day-to-day variation) were significantly lower in Gla300/Glu than IDegAsp. The postprandial glucose levels 2 h after breakfast were significantly lower in IDegAsp than Gla300/Glu (Table 2). Figure 2 shows the average daily glucose profiles measured by FGM for each 3-day study period. IDegAsp lowered the postprandial glucose levels 2 h after breakfast better than Gla300/Glu; levels were almost the same after lunch and supper. IDegAsp lowered nocturnal (00.00–06.00 hours) glucose levels better than Gla300/Glu.

Diurnal variation of serum albumin and the correlation between hypoglycemia and serum albumin in patients treated with IDegAsp

In the present study, we investigated the factors associated with hypoglycemia. We measured serum albumin at 06.00 and 21.00 hours on day 2 and day 12 after FGM attachment, respectively. Figure S1 shows diurnal variation of serum albumin and the correlation between hypoglycemia and serum albumin in patients treated with IDegAsp. After changing to Gla300/Glu, the Gla300 dosage was 11.0 U/day (8.0–15.5 U/day) and Glu dosage was 4.0 U/day (3.0–4.3 U/day). The ultra-rapid-acting insulin Glu dosage was significantly lower than the total dosage of Asp (P = 0.004). The Asp dosage at supper was conversely significantly lower than the Glu dosage (P = 0.0009).
negative correlation with the mean percentage of time of nocturnal (00.00–06.00 hours) hypoglycemia (Figure 3a); however, at 21.00 hours, there was no significant correlation with daytime (06.00–24.00 hours) hypoglycemia (Figure 3c). In contrast, such a negative correlation was not shown in the treatment period with Gla300/Glu (Figure 3b,d).

Table 2 | Flash glucose monitoring parameters of glucose levels in patients with insulin degludec/aspart and glargine 300 U/mL/insulin glulisine

|                      | IDegAsp      | Gla300/Glu   | P-value*   |
|----------------------|--------------|--------------|------------|
| Mean percentage of time within the target glucose range, 70–180 mg/dL (%) | 73.1 (69.4–81.1) | 84.2 (80.2–93.1) | 0.001*     |
| Mean percentage of time with hyperglycemia, ≥180 mg/dL (%) | 9.2 (4.4–13.6) | 7.6 (2.7–12.0) | 0.522      |
| 24-h SD (mg/dL)      | 42.5 (38.6–45.9) | 35.5 (31.1–40.6) | 0.014*     |
| 24-h Mean value (target glucose level 100 mg/dL) | 7.5 (5.6–9.3) | 4.7 (3.1–6.3) | <0.001*    |
| 24-h CV (%)          | 0.4 (0.3–0.4) | 0.3 (0.3–0.4) | <0.001*    |
| 00.00–06.00 hours CV (%) | 0.3 (0.3–0.3) | 0.3 (0.3–0.3) | 0.001*     |
| MAGE (mg/dL)         | 92.5 (75.5–100.1) | 75.1 (67.9–91.1) | 0.008*     |
| MODD (mg/dL)         | 24.4 (20.9–35.9) | 21.6 (18.1–24.6) | 0.002*     |
| 24-h mean glucose level (mg/dL) | 118.7 (106.1–123.9) | 119.6 (101.3–123.0) | 0.522      |
| 00:00–6:00 hours mean glucose level (mg/dL) | 113.9 (101.3–123.0) | 114.3 (109.2–123.1) | 0.571      |
| 08:00–12:00 hours mean glucose level (mg/dL) | 122.8 (105.3–128.0) | 121.2 (116.3–131.7) | 0.522      |
| 12:00–24:00 hours mean glucose level (mg/dL) | 120.3 (107.7–128.6) | 121.5 (112.6–133.5) | 0.701      |
| Preprandial glucose level at breakfast (mg/dL) | 100.7 (93.8–112.4) | 105.0 (90.7–133.4) | 0.165      |
| Preprandial glucose level at lunch (mg/dL) | 119.8 (103.8–129.1) | 126.3 (112.3–143.3) | 0.360      |
| Preprandial glucose level at supper (mg/dL) | 111.5 (103.8–125.7) | 115.3 (101.2–139.9) | 0.784      |
| Postprandial glucose level 2 h after breakfast (mg/dL) | 99.2 (146.8–160.8) | 146.4 (159.2–172.3) | 0.028*     |
| Postprandial glucose level 2 h after lunch (mg/dL) | 1380 (1552–1746) | 1282 (1467–1670) | 0.216      |
| Postprandial glucose level 2 h after supper (mg/dL) | 1392 (1083–1651) | 1312 (1030–1488) | 0.294      |
| Mean percentage of time with hypoglycemia, <70 mg/dL (%) | 14.4 (4.4–23,5) | 2.1 (0.0–9.4) | 0.012*     |
| Mean percentage of time with clinically important hypoglycemia, <54 mg/dL (%) | 1.9 (0.0–5.6) | 0.0 (0.0–0.2) | 0.036*     |
| Mean percentage of time with nocturnal hypoglycemia, <70 mg/dL (%) | 8.9 (3.1–11.8) | 0.5 (0.0–5.9) | 0.007*     |
| Mean percentage of time with daytime hypoglycemia, <70 mg/dL (%) | 3.2 (0.5–11.4) | 0.7 (0.0–20.0) | 0.073      |

Values are expressed as median (interquartile range). *Data were compared using Wilcoxon’s signed rank test. A P-value of <0.05 was considered significant. CV, coefficient of variation; Gla300, glargine 300 U/mL; Glu, insulin glulisine; IDegAsp, insulin degludec/aspart; MAGE, mean amplitude of glycemic excursion; MODD, mean of daily difference; SD, standard deviation of the glucose levels.

Figure 2 | The 24-h mean glucose levels on the basis of the flash glucose monitoring system for 3 days. Solid line indicates basal–bolus therapy with insulin glargine 300 U/mL and insulin glulisine (Gla300/Glu). Dashed line indicates twice-daily injection therapy with insulin degludec/aspart (IDegAsp).
DISCUSSION

The results of the present study clarified that treatment with Gla300/Glu was superior to that with IDegAsp in terms of efficacy and safety through the use of the FGM system. We used the mean percentage of time with a glucose range of 70–180 mg/dL as an index of effectiveness, and the result was that treatment with Gla300/Glu was significantly more frequent in this range than treatment with IDegAsp. The reason for this was that the glucose levels of 06.00–24.00 hours were almost in the target glucose range of 70–180 mg/dL levels for both treatments; however, the glucose levels of 00.00–06.00 hours were lower after treatment with IDegAsp than with Gla300/Glu (Figure 2). As long-acting insulin contributed to nocturnal glucose levels, it was thought that this result was caused by the difference in pharmacodynamics between IDeg and Gla300. Intra- and interday variability in the glucose-lowering effect of IDeg was significantly lower than that of Gla300. Another study compared the efficacy between IDeg and Gla300, and reported that the standard deviation, an index of diurnal variation in glucose levels with IDeg, was significantly higher than that of Gla300, with IDeg having peak action between 8 and 12 h after injection. In the present study, diurnal variation in glucose level after treatment with Gla300/Glu was significantly lower than that after treatment with IDegAsp, indicated by the 24-h mean value, 24-h CV, mean amplitude of glycemic excursion and mean of the daily difference, an index of day-to-day variation. During the insulin titration in treatment period 1, the preprandial glucose level at breakfast and supper was achieved at 100–130 mg/dL; however, hypoglycemia was significantly higher during the analysis period of IDegAsp than during the analysis period of Gla300/Glu. It is imperative to focus on the treatment with IDegAsp twice-daily, as it is associated with the risk of hypoglycemia unawareness. Severe and nocturnal hypoglycemia has been recognized as an important limiting factor for enhancing treatment of diabetes. Among diabetes specialists, 80% they are unable to proactively treat the disease.
because of the risk of hypoglycemia\textsuperscript{11}. Another study showed that 50–59\% of patients did not report hypoglycemia to doctors, and 49–64\% of patients developed hypoglycemia that they were unaware of \textsuperscript{30}. Furthermore, when severe hypoglycemia occurs, a hazard ratio of death due to cardiovascular events is reported to be 2.68\textsuperscript{31}; thus, treatment that does not result in hypoglycemia is very important for diabetes patients. When hypoglycemia occurs, we might hesitate to increase the dose of treatment drugs, but basal–bolus treatment using Gla300/Glu has a lower risk of hypoglycemia, which enables more aggressive treatment and is easier to titrate with less variation. In the present study, the IDeg contained in IDegAsp was switched to Gla300 at the same dosage. The Asp, ultra-rapid-acting insulin, contained in IDegAsp, was switched to Glu at the same dosage at the time of switching, and the dosage of Glu was significantly lower than the total dosage of Asp. Furthermore, Asp dosage at supper was conversely significantly lower than Glu dosage, and as a result, we titrated the dosage of Glu so that the postprandial glucose level 2 h after supper was 130–150 mg/dL. This is because IDegAsp is premixed insulin, which makes it impossible to individually adjust long-acting insulin and ultra-rapid-acting insulin. It is also difficult to achieve both prepandial and postprandial glucose levels moderately. However, treating patients with a twice-daily injection using only one type of insulin has the advantage of reducing hypoglycemia risk in patients using a dose that is not prescribed\textsuperscript{11}. The treatment of basal–bolus of Gla300/Glu uses two types of insulin, which increases this risk\textsuperscript{11}; however, flexibility in treatment is allowed, as bolus insulin can be adjusted according to the largest meal\textsuperscript{32}. As the Asp component of IDegAsp was injected before breakfast in treatment period 1, the treatment with IDegAsp allowed significantly lower postprandial glucose levels 2 h after breakfast than that with Gla300/Glu. In treatment period 2, Glu was injected before supper and the largest meal, and the postprandial glucose levels 2 h post-supper were lower in Gla300/Glu; no significant difference was seen. In addition, the Asp dosage at supper was significantly lower than Glu dosage. IDeg had a peak effect 8–12 h after injection,\textsuperscript{33} whereas IDeg/Asp injected at breakfast showed its postprandial peak effect 2 h after supper, and overlapped with the hypoglycemic effect of Asp. For this reason, it is considered that the dosage of Asp containing IDeg/Asp at supper was significantly smaller than Glu, and the postprandial glucose level 2 h post-supper became comparable between the two treatments.

In the present study, the cause of significant difference in the hypoglycemic index might be because of the difference in the mechanism of action of IDeg and Gla300. We previously reported that IDeg and serum albumin values have a negative correlation\textsuperscript{33}. IDeg is an insulin that binds irreversibly to albumin. After subcutaneous administration, IDeg forms soluble long-term stable multi-hexamers, and temporarily remains in the subcutaneous tissue of the injection site. As the monomer gradually dissociates from the multi-hexamer, it slowly and continuously transfers into circulation\textsuperscript{34}. This is a major explanatory factor for the stability and durability of IDeg; however, its binding to albumin, as one of the multiple factors, explains its stability and durability\textsuperscript{34}. Gla300, in contrast, does not bind to albumin\textsuperscript{35,36}. In circulation, IDeg binds to albumin, reaches its target tissue, and IDeg off the albumin binds to the insulin receptor and exerts a hypoglycemic effect\textsuperscript{37,38}. The association constant (Ka = B / [F × HSAimm]) has been determined for IDeg, with B/F as the ratio between bound insulin and free insulin, and HSAimm as the total concentration of immobilized albumin\textsuperscript{39}. Albumin has a diurnal variation with the maximum value in the daytime, and the lowest value during the night\textsuperscript{15}. In the present study, albumin was also significantly lower at 06.00 hours than at 21.00 hours (Figure S1). As Ka is a constant, it maintains a fixed value. Therefore, as serum albumin decreases, free insulin increases and bound insulin decreases to maintain the constant level. As free insulin binds to the insulin receptor and administers its effect, the serum albumin at 06.00 hours and nocturnal hypoglycemia have shown a negative correlation during the treatment period of IDegAsp. In contrast, there was no significant difference between the serum albumin at 21.00 hours and the daytime hypoglycemia, with the serum albumin significantly higher than at 06.00 hours. There was a significant difference in the mean percentage of time with daily hypoglycemia between the two treatment periods, but there was no significant difference in the mean percentage of time with daytime hypoglycemia. From this, the difference between IDeg/Asp and Gla300/Glu is thought to be different between the expression of IDeg and Gla300 nocturnal hypoglycemia. When IDegAsp is administered to elderly people, malnourished patients, patients with nephrotic syndrome due to renal disease and patients with decreased albumin synthesis due to liver disease, it is necessary to consider the risk of hypoglycemia. Insulin detemir is also insulin that binds to albumin; however, free insulin of insulin detemir has a negative correlation with free fatty acid (FFA)/serum albumin,\textsuperscript{39} and diabetes patients are known to have increased FFA, especially at night\textsuperscript{40}. Thus, hypoalbuminemia at night and high FFA might increase the risk of hypoglycemia during the treatment period using IDegAsp. Participants in the present study had mild-to-moderate renal impairment with a median estimated glomerular filtration rate of 55.8 mL/min/1.73 m\textsuperscript{2} \textsuperscript{41}, the influence of hypoalbuminemia due to diabetic nephropathy was also considered. However, it has been reported that pharmacokinetics of IDeg and Asp were not affected by renal impairment\textsuperscript{41,42}; hypoglycemia of IDegAsp is thought to be related to hypoalbuminemia.

The present study had several limitations. The first limitation is that those patients who had already used IDegAsp experienced poor glycemic control. After hospitalization, we titrated the dosage of IDegAsp, setting the glucose target range before breakfast and supper, and switched to Gla300 with the same dosage of IDeg contained in IDegAsp. To achieve the equivalent glycemic control level for patients using Gla100, the required dosage of IDeg is possible in
fewer dosages. If the same applies to Glu300, it is necessary to increase the dosage of Glu300 for IDeg. Therefore, in the treatment algorithm, if the targeted blood glucose control is not obtained by the patient being treated with basal insulin, injection of the premix preparation twice a day is recommended, or injection of ultra-rapid-acting insulin at the time of the largest meal is carried out. To prove which of the two treatments is effective and safe, it is necessary to switch to IDegAsp for patients with insufficient glycemic control by basic insulin treatment and switch to Glu300/Glu, or vice versa (cross-over study). In the present study, it was necessary to titrate IDegAsp, Glu300 and Glu to match the target blood glucose level. In real-world treatment, it is necessary to carry out a multicentered, randomized, double-blinded, parallel group study to compare efficacy and safety, long-term blood glucose control, and the risk of cardiovascular events and cardiovascular death.

The second limitation of the study is that a negative correlation was found between the diurnal variation of serum albumin value and 24-h hypoglycemia and nocturnal hypoglycemia. In addition, a measure of free insulin unbound to albumin was not carried out. As a method for measuring free insulin, adding polyethylene glycol and mixing is known; however, it is a rather complicated procedure, and the inspection result might become unstable. The change in ratio of free insulin to bound insulin by serum albumin value was predicted using the formula of Ka; however, a measure of free insulin and FFA is also important.

The relatively small sample size is another limitation, in addition to the lack of generalizability to other populations and lack of same-time comparison of different insulin types.

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DISCLOSURE
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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | The diurnal variation of serum albumin on day 2 after fasting glucose monitor (FGM) attachment. Serum albumin at 06.00 hours was 3.7 g/dL (3.6–3.9 g/dL), and at 21.00 hours was 3.8 g/dL (3.7–4.0 g/dL), the former was significantly lower than the latter (P = 0.013). Statistical analysis was carried out with Wilcoxon’s signed rank test. A P-value of <0.05 was considered significant.