

# Comparison of morning basal + 1 bolus insulin therapy (insulin glulisine + insulin glargine 300 U/mL vs insulin lispro + insulin glargine biosimilar) using continuous glucose monitoring: A randomized crossover study

Soichi Takeishi\* ID, Hiroki Tsuboi, Shodo Takekoshi

Department of Diabetes, General Inuyamachuo Hospital, Inuyama-city, Aichi, Japan

## Keywords

Continuous glucose monitoring, Long-acting insulin, Ultra-rapid-acting insulin

## \*Correspondence

Soichi Takeishi  
Tel.: +81-568-62-8111  
Fax: +81-568-62-9289  
E-mail address:  
souichi19811225@yahoo.co.jp

*J Diabetes Investig* 2018; 9: 91–99

doi: 10.1111/jdi.12661

## Clinical Trial Registry

University Medical Information Network  
UMIN000022094

## ABSTRACT

**Introduction:** We compared the effects of morning administration of insulin glulisine + insulin glargine 300 U/mL (G + G300) with that of insulin lispro + insulin glargine biosimilar (L + GB).

**Materials and Methods:** A total of 30 patients with type 2 diabetes who wore a continuous glucose monitoring device on admission after glucose levels were stabilized by morning long-acting and ultra-rapid-acting insulins were randomly allocated to groups who received G + G300 on days 1 and 2, and the same dose L + GB on days 3 and 4, or vice versa. Data collected on days 2 and 4 (mean amplitude of glycemic excursion, mean of daily differences: all days) were analyzed. Insulin was injected at 08.00 h. A day was defined as the period from 08.00 h one day, to 08.00 h the next day. Test meals were given.

**Results:** Increased post-breakfast glucose level, post-breakfast glucose gradient, mean glucose level, standard deviation and M-value (24 h, 00.00–06.00 h), mean amplitude of glycemic excursion, and mean of daily differences were significantly lower in patients taking G + G300 than those taking L + GB ( $P \leq 0.0001$ – $0.04$ ). The area over the glucose curve ( $<70$  mg/dL) was not significantly different between groups. Pre-lunch – pre-breakfast glucose levels were significantly lower in patients taking L + GB than those taking G + G300 ( $P < 0.0001$ ). The difference in the highest post-breakfast glucose level between groups ( $\Delta = G + G300 - L + GB$ ) was significantly correlated to 24-h mean glucose level ( $r = 0.40$ ,  $P = 0.03$ ).

**Conclusions:** Compared with L + GB, G + G300 decreases post-breakfast glucose level reducing rate of rise of that, nocturnal and 24-h glucose variability and level without causing hypoglycemia, and daily variance.

## INTRODUCTION

A relationship between nocturnal hypoglycemia and sudden death has been suggested.<sup>1</sup> As per the Somogyi phenomenon, nocturnal hypoglycemia causes an increase in the difference between pre- and post-breakfast glucose levels.<sup>2</sup> Thus, hypoglycemia and increased glycemic variability occur at the same

time in the Somogyi phenomenon. Large clinical studies have shown that hypoglycemia and glycemic variability are associated with mortality in patients with diabetes mellitus.<sup>3–5</sup> We wanted to predict the Somogyi phenomenon, in which hypoglycemia and increased glycemic variability occur concomitantly, and we have reported that major increases between pre- and post-breakfast glucose levels might predict nocturnal hypoglycemia in type 2 diabetes patients.<sup>6</sup>

Received 30 November 2016; revised 13 March 2017; accepted 21 March 2017

When the Somogyi phenomenon is suspected, reducing both nocturnal hypoglycemia and increases between pre- and post-breakfast glucose levels, and controlling pre-breakfast glucose levels properly, is necessary.<sup>1,4,7</sup> We consider that morning long-acting insulin reducing nocturnal hypoglycemia and ultra-rapid-acting insulin reducing increases between pre- and post-breakfast glucose levels accurately are useful. Adding one ultra-rapid-acting insulin to long-acting insulin is recommended in the American Diabetes Association's guideline.<sup>8</sup> Therefore, this treatment is thought to be useful as an insulin therapy that is injected in the morning only.

Regarding reducing nocturnal hypoglycemia, nocturnal and overall hypoglycemia were significantly reduced in patients taking insulin glargine 300 U/mL (glargine 300 U/mL), as opposed to insulin glargine 100 U/mL (glargine 100 U/mL) in the Every Day in the Life of Patients with Diabetes Japan 2 trial.<sup>9</sup> Therefore, glargine 300 U/mL is thought to be useful as a long-acting insulin in reducing nocturnal hypoglycemia.

Regarding increases between pre- and post-breakfast glucose levels, post-breakfast glucose levels are often increased to a greater extent than post-lunch or post-supper glucose levels, and can cause increased glycemic variability over 24 h.<sup>10</sup> The glucose level after the first meal (breakfast),<sup>11</sup> and sometimes the Somogyi phenomenon<sup>2</sup> and the dawn phenomenon,<sup>12</sup> are the main causes of increased morning glucose levels. However, the Somogyi phenomenon is mainly caused by nocturnal hypoglycemia. Differentiating the Somogyi phenomenon from the dawn phenomenon is difficult, unless whether nocturnal hypoglycemia is occurring or not is known. In any case the following are necessary in order to reduce increases in post-breakfast glucose levels: (i) adjusting treatment to avoid nocturnal hypoglycemia when nocturnal hypoglycemia causes increased morning glucose levels; and (ii) decreasing post-breakfast glucose levels directly by adjusting treatment. Regarding (ii), knowledge of a patient's morning glycemic variability status is important for improving increased glycemic variability over 24 h. We focused on using patient characteristics to predict morning glycemic variability, and we have reported the relationship between patient characteristics and morning glycemic variability in a previous study.<sup>13</sup> In that study, higher age and lower body mass index (BMI) were observed to be associated with higher highest increased post-breakfast glucose levels. Furthermore, longer durations of diabetes and lower BMI were correlated with a higher post-breakfast glucose gradient. Therefore, we believe that treatments that decrease post-breakfast glucose levels in elderly diabetes patients, as well as a delay or reduction in the increases in post-breakfast glucose levels of diabetes patients with low BMI values, should be considered. Specifically, we believe that insulin glulisine (glulisine), which has a fast onset of action, is beneficial in this clinical scenario. It has been reported that switching to glulisine from another ultra-rapid-acting insulin with almost the same dose improved postprandial glucose levels and glycosylated hemoglobin levels.<sup>14</sup>

Therefore, we hypothesized that glulisine is useful as the ultra-rapid-acting insulin in reducing increases between pre- and post-breakfast glucose levels.

Thus, a combination therapy of glargine 300 U/mL (considered to reduce nocturnal hypoglycemia and pre-breakfast glucose levels) and glulisine (post-breakfast glucose level, the rate of rise of which was reduced, decreases, because the effects of the difference of insulin concentration on glulisine were faster and stronger) administered in the morning might be beneficial. We compared the effects of morning administration of insulin glulisine + insulin glargine 300 U/mL (G + G300) with that of insulin lispro + insulin glargine biosimilar (L + GB) using continuous glucose monitoring (CGM) in a randomized cross-over study.

## METHODS

### Study design and patient selection

Fasting plasma glucose (FPG) of the glargine 100 U/mL group in the Every Day in the Life of Patients with Diabetes Japan 2 trial<sup>9</sup> was  $133.84 \pm 34.73$  mg/dL. The number of individuals for whom a 30% alteration of the mean FPG could be detected with an  $\alpha$  value of 0.05 and with a statistical power of 80% was 13. We assumed that 10% of those recruited might drop out of the trial, and therefore determined the total number of participants required to be 15. The glucose level of the insulin lispro (lispro) group of patients with type 2 diabetes in the clinical trial investigating efficacy and safety of glulisine reported by Morishita<sup>15</sup> was  $162.51 \pm 58.94$  mg/dL, 1 h post-breakfast. The number of individuals for whom a 30% alteration of the mean 1-h post-breakfast glucose level could be detected with an  $\alpha$  value of 0.05 and with a statistical power of 80% was 24. We assumed that 10% of those recruited might drop out of the trial, and therefore determined the total number of participants required to be 27. From the above, the necessary number of samples was determined to be 27.

A total of 30 patients with type 2 diabetes treated with basal insulin for 3 months or longer were investigated. The patients who wore a CGM device (Medtronic ipro2; Medtronic Minimed, Northridge, CA, USA) on admission were randomly allocated to separate groups (each group included 15 patients) who received G + G300 on days 1 and 2, and the same dose of L + GB on days 3 and 4, or who received L + GB on days 1 and 2, and the same dose of G + G300 on days 3 and 4.<sup>16</sup> Patients were allocated to groups using a random number table, and the study design was continuous and prospective. When basal insulin was insulin degludec, it was changed to another long-acting insulin after enrollment. All patients' insulin regimens were unified into morning long-acting insulin and ultra-rapid-acting insulin after enrollment. Insulin was titrated to stabilize FPG not exceeding 180 mg/dL for 3 days, and then the CGM device was attached. Glucose levels were measured for four consecutive days by the CGM device, and data collected on days 2 and 4 (mean amplitude of glycemic excursion [MAGE],<sup>17</sup> mean of daily differences [MODD]<sup>18</sup> and average

daily risk range<sup>19</sup>: days 1 and 2, and days 3 and 4) were analyzed. Insulin was injected at 08.00 h. A day was defined as the period from 08.00 h 1 day, to 08.00 h the next day. Regarding other diabetes treatments,  $\alpha$ -glucosidase inhibitors, rapid-acting insulin secretagogues and glucagon-like peptide-1 receptor agonists were discontinued after enrollment. All other treatments were continued during the CGM measurement period. The participants received test meals consisting of the same nutrients and equivalent caloric intake during the CGM measurement period. The total caloric intake for participants was defined by the attending physicians, as 1,440 kcal, 1,600 kcal or 1,840 kcal per day to accommodate differences in physique among the participants.<sup>20</sup> Test meals were given to each patient based on the recommendation of the Japan Diabetes Society (component ratio of calories [carbohydrates 60%, proteins 18% and lipids 22%; breakfast 30%; lunch 35%; supper 35%]), irrespective of differences in physique during the CGM measurement period. Physical activity at admission to the study was 1.5 metabolic equivalents based on the analysis of baseline data. Patients with severe renal dysfunction (serum creatinine level  $\geq 2.0$  mg/dL) or judged to be unsuitable for participation for medical reasons were excluded from this study.

The study protocol was approved by the ethical committee of General Inuyamachuo Hospital (authorization no. I, 2016), and was registered in a clinical trial database with the University Medical Information Network (no. UMIN000022094).

### Statistical analysis

The CGM end-point included M-value (24 h [target glucose level 100], 08.00–12.00 h [target glucose level = 120], 12.00–24.00 h [target glucose level 120], 00.00–06.00 h [target glucose level 90]),<sup>21</sup> MAGE (MAGE of increased glucose levels [MAGE+], MAGE of decreased glucose levels [MAGE-]),<sup>17</sup> MODD, average daily risk range, mean glucose level (24 h, 08.00–12.00 h, 12.00–24.00 h, 00.00–06.00 h), standard deviation (SD)<sup>22</sup> (24 h, 08.00–12.00 h, 12.00–24.00 h, 00.00–06.00 h), preprandial glucose level (lunch – breakfast),<sup>23</sup> highest postprandial glucose level within 3 h after each meal (highest glucose level), time from start of meal to the highest postprandial glucose level (highest glucose time), difference between preprandial and highest postprandial glucose level for each meal (increased glucose level), postprandial glucose gradient, area under the glucose curve (AUC;  $\geq 180$  mg/dL) within 3 h of each meal, AUC ( $\geq 0$  mg/dL) in 24 h and area over the glucose curve (AOC;  $< 70$  mg/dL) (24 h, 08.00–12.00 h, 00.00–06.00 h).<sup>24</sup>

Data are shown as median (interquartile range). Statistical analysis was carried out with Wilcoxon signed-rank test. A *P*-value of  $< 0.05$  was considered significant.

## RESULTS

### Patient characteristics

Table 1 shows the patients' characteristics. The study included 13 men and 17 women. The baseline characteristics were as

follows: age, 71.5 years (65.0–80.0 years); duration of diabetes, 15.0 years (10.0–20.0 years); BMI, 25.3 kg/m<sup>2</sup> (22.4–26.7 kg/m<sup>2</sup>); glycosylated hemoglobin, 8.3% (7.6–9.3%); C-peptide immunoreactivity, 1.2 ng/mL (0.8–1.7 ng/mL); FPG level, 140.5 mg/dL (111.8–190.0 mg/dL); C-peptide index, 0.7 (0.6–1.1); urine C-peptide immunoreactivity, 23.4  $\mu$ g/day (12.5–48.9  $\mu$ g/day); basal insulin dose, 12.0 U/day (8.0–24.0 U/day); bolus insulin dose, 6.0 U/day (4.0–7.5 U/day); total insulin dose, 18.0 U/day (14.0–30.0 U/day); and basal/total insulin dose ratio 70.3% (62.5–75.0%; Table 1).

### Primary outcomes

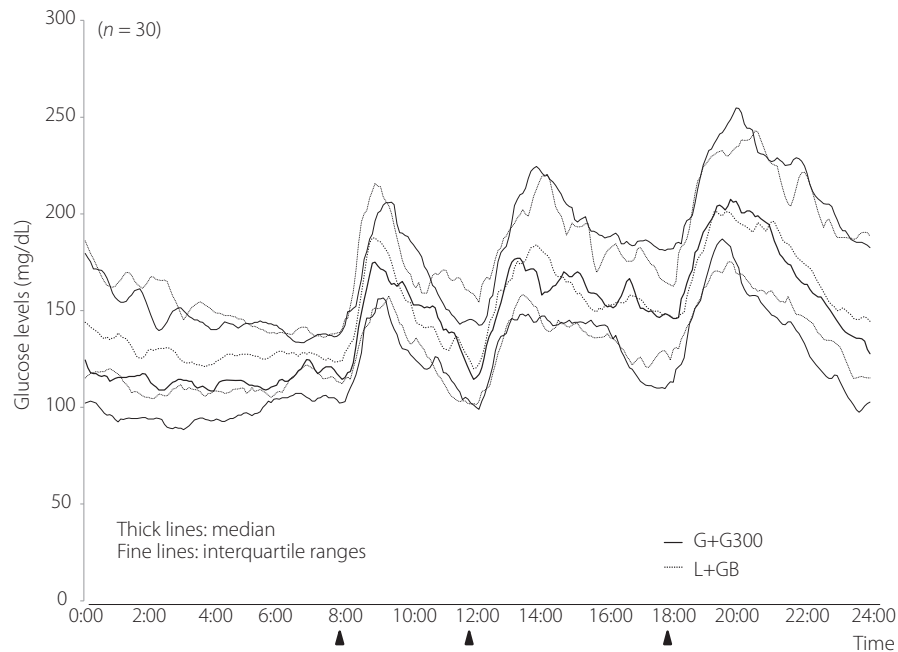
Figure 1 shows the glycemic variability over 24 h of CGM in all of the patients.

The M-values (24 h, 00.00–06.00 h, 08.00–12.00 h) were significantly lower in patients taking G + G300 than those taking L + GB (*P* = 0.003, *P* = 0.0004, *P* = 0.04, respectively). MAGE, MAGE+ and MAGE- were significantly lower in patients taking G + G300 than those taking L + GB (*P* = 0.0001, *P* < 0.0001, *P* = 0.0004, respectively). MODD was significantly lower in patients taking G + G300 than those taking L + GB (*P* = 0.004). Mean glucose levels (24 h, 00.00–06.00 h, 12.00–24.00 h) were significantly lower in patients taking G + G300 than those taking L + GB (*P* = 0.0002, *P* = 0.0002, *P* = 0.01, respectively). SDs (24 h, 00.00–06.00 h, 08.00–12.00 h) were significantly lower in patients taking G + G300 than those taking L + GB (*P* = 0.008, *P* = 0.0003, *P* = 0.009, respectively).

**Table 1** | Baseline characteristics

Characteristic	Value
<i>n</i> (male/female)	30 (13/17)
Age (years)	71.5 (65.0–80.0)
Duration of diabetes (years)	15.0 (10.0–20.0)
BMI (kg/m <sup>2</sup> )	25.3 (22.4–26.7)
HbA1c, NGSP (%)	8.3 (7.6–9.3)
GA (%)	21.8 (19.2–24.0)
CPR (ng/mL)	1.2 (0.8–1.7)
FPG (mg/dL)	140.5 (111.8–190.0)
CPI	0.7 (0.6–1.1)
U-CPR ( $\mu$ g/day)	23.4 (12.5–48.9)
Basal insulin dose (U/day)	12.0 (8.0–24.0)
Bolus insulin dose (U/day)	6.0 (4.0–7.5)
Total insulin dose (U/day)	18.0 (14.0–30.0)
Basal/Total insulin dose ratio (%)	70.3 (62.5–75.0)
Sulfonylurea agent, <i>n</i> (%)	3 (10.0)
Biguanide agent, <i>n</i> (%)	19 (63.3)
Thiazolidine, <i>n</i> (%)	3 (10.0)
DPP4 inhibitor, <i>n</i> (%)	21 (70.0)
SGLT 2 inhibitor, <i>n</i> (%)	8 (26.7)

Data are shown as median (interquartile range). BMI, body mass index; CPI, C-peptide index; CPR, C-peptide immunoreactivity; DPP, dipeptidyl-peptidase; FPG, fasting plasma glucose; GA, glycoalbumin; HbA1c, glycosylated hemoglobin; SGLT, sodium glucose co-transporter; U-CPR, urine C-peptide immunoreactivity.



**Figure 1** | The graph shows glucose variability over 24 h on continuous glucose monitoring (CGM) in patients during treatment with insulin glulisine + insulin glargine 300 U/mL (G + G300) or insulin lispro + insulin glargine biosimilar (L + GB). Glucose levels were calculated from the value of CGM on the second day after the start of each therapy. Data are shown as median (thick lines) and interquartile ranges (fine lines).

Pre-lunch – pre-breakfast glucose level was significantly lower in patients taking L + GB than those taking G + G300 ( $P \leq 0.0001$ ). The highest post-breakfast glucose level was significantly lower in patients taking G + G300 than those taking L + GB ( $P \leq 0.0001$ ). Increased post-breakfast glucose level was significantly lower in patients taking G + G300 than those taking L + GB ( $P = 0.0001$ ). The post-breakfast glucose gradient was significantly lower in patients taking G + G300 than those taking L + GB ( $P = 0.0007$ ). AUC ( $\geq 180$  mg/dL) within 3 h after breakfast was significantly lower in patients taking G + G300 than those taking L + GB ( $P = 0.001$ ). The AUC ( $\geq 0$  mg/dL) in 24 h was significantly lower in patients taking G + G300 than those taking L + GB ( $P = 0.0002$ ). The area over the glucose curve ( $< 70$  mg/dL) was not significantly different between groups (Table 2).

The difference in the highest post-breakfast glucose levels between groups ( $\Delta = G + G300 - L + GB$ ) was significantly correlated to AUC in 24 h ( $r = 0.39$ ,  $P = 0.03$ ; Spearman's rank correlation coefficient).

## DISCUSSION

The results of the present study suggest that the combination therapy of G + G300 decreases post-breakfast glucose level reducing rate of rise of that, nocturnal and 24-h glucose variability and level without causing hypoglycemia, and daily variance. Figure 2 shows a schematic diagram of the estimated insulin concentration in both G + G300 and L + GB, to

investigate the basis of the results obtained from the present study.

The finding that the highest post-breakfast glucose level, increased post-breakfast glucose level, post-breakfast glucose gradient and AUC ( $\geq 180$  mg/dL) within 3 h after breakfast were significantly lower in patients taking G + G300 than those taking L + GB was thought to be caused by the difference of insulin concentration between glulisine and lispro (part 1 of Figure 2) because of the rapid onset of action of glulisine. Namely, we believe that post-breakfast glucose levels decreased with the reduced rate of rise, because glulisine was affected faster and stronger by the difference (part 1 of Figure 2). In fact, it has been reported that time to 50% serum insulin concentration of glulisine was significantly earlier than that of lispro or insulin aspart.<sup>25</sup> The onset of glulisine's effect has been suggested to be faster than the other ultra-rapid-acting insulins, and this is supported by the results of the present study. The result that the difference in the highest post-breakfast glucose level between groups ( $\Delta = G + G300 - L + GB$ ) was significantly correlated to AUC in 24 h, suggests that as well as reducing the highest post-breakfast glucose levels, glulisine also produces a greater reduction in glucose levels measured over a 24-h period. Conversely, the result that pre-lunch – pre-breakfast glucose levels were significantly lower in patients taking L + GB than those taking G + G300 was thought to be due to the difference in serum insulin concentration between glulisine and lispro (part 2 of Figure 2), because serum concentrations

**Table 2** | Parameters of glucose variability in patients treated with insulin glulisine + insulin glargine 300 U/mL or insulin lispro + insulin glargine biosimilar

	Glulisine + glargine 300 U/mL	Lispro + glargine biosimilar	P
24-h M-value (target glucose level 100 mg/dL)	8.4 (4.4–22.4)	9.7 (6.2–31.9)	0.003
00.00–06.00 h M-value (target glucose level 90 mg/dL)	1.5 (0.4–11.2)	5.1 (1.7–22.4)	0.0004
08.00–12.00 h M-value (target glucose level 120 mg/dL)	2.1 (0.7–5.9)	2.6 (1.2–7.4)	0.04
12.00–24.00 h M-value (target glucose level 120 mg/dL)	4.6 (2.4–14.9)	4.7 (2.7–22.5)	0.05
MAGE (mg/dL)	47.6 (38.2–55.7)	53.2 (46.1–86.8)	0.0001
MAGE of increased glucose levels (mg/dL)	48.3 (38.9–56.9)	54.1 (47.4–86.9)	<0.0001
MAGE of decreased glucose levels (mg/dL)	46.7 (37.9–54.6)	53.8 (44.3–87.3)	0.0004
Mean of daily difference (mg/dL)	24.6 (16.4–31.1)	25.2 (19.6–39.0)	0.004
Average daily risk range	14.4 (9.0–22.4)	17.3 (8.9–24.5)	0.26
24-h mean glucose level (mg/dL)	151.0 (128.1–167.7)	153.4 (142.2–192.4)	0.0002
00.00–06.00 h mean glucose level (mg/dL)	113.1 (93.4–137.8)	132.1 (115.4–168.8)	0.0002
08.00–12.00 h mean glucose level (mg/dL)	149.2 (126.9–163.6)	150.3 (135.2–166.8)	0.21
12.00–24.00 h mean glucose level (mg/dL)	164.1 (137.9–202.6)	166.8 (156.2–211.0)	0.01
24-h standard deviation (mg/dL)	33.4 (27.2–40.3)	33.5 (27.3–55.0)	0.008
00.00–06.00 h SD (mg/dL)	8.5 (6.1–13.9)	10.8 (8.7–18.4)	0.0003
08.00–12.00 h SD (mg/dL)	20.3 (16.5–26.5)	25.8 (18.0–32.7)	0.009
12.00–24.00 h SD (mg/dL)	28.1 (21.5–33.6)	30.4 (21.0–38.8)	0.29
Preprandial glucose level (mg/dL)			
Lunch – breakfast	7.5 (–10.5 to 29.3)	–4.0 (–32.0 to 5.8)	<0.0001
Highest postprandial glucose level within 3 h after each meal (mg/dL)			
Breakfast	173.5 (160.6–192.8)	198.0 (165.8–217.0)	<0.0001
Lunch	193.5 (167.5–232.5)	200.0 (173.8–241.8)	0.14
Supper	212.8 (187.0–251.0)	214.5 (198.0–258.8)	0.5
Time from start of meal to the highest postprandial glucose level (min)			
Breakfast	75.0 (51.3–98.8)	62.5 (50.0–85.0)	0.98
Lunch	97.5 (71.3–158.8)	100.0 (70.0–125.0)	0.34
Supper	102.5 (81.3–140.0)	97.5 (71.3–158.8)	0.76
Differences between preprandial and highest postprandial glucose level for each meal (mg/dL)			
Breakfast	47.0 (29.5–71.3)	66.0 (47.5–95.5)	0.0001
Lunch	77.5 (47.3–100.8)	76.0 (48.3–104.8)	0.31
Supper	69.0 (33.8–89.8)	61.5 (40.3–101.0)	0.98
Postprandial glucose gradient (mg/dL min)			
Breakfast	0.73 (0.31–1.11)	1.26 (0.66–1.72)	0.0007
Lunch	0.68 (0.39–1.01)	0.79 (0.52–1.04)	0.43
Supper	0.55 (0.35–1.05)	0.59 (0.36–0.98)	0.4
AUC ( $\geq 180$ mg/dL) within 3 h after each meal (mg min/dL)			
Breakfast	0.0 (0.0–828.1)	690.0 (0.0–4,338.1)	0.001
Lunch	180.6 (0.0–5,804.1)	1,219.4 (0.0–7,058.8)	0.13
Supper	4,009.4 (251.3–13,306.9)	3,159.9 (343.1–12,530.6)	0.86
24-h AUC, $\geq 0$ mg/dL (mg min/dL)	217,386.7 (184,660.1–241,610.1)	220,806.8 (204,782.1–277,096.4)	0.0002
24-h AOC, $<70$ mg/dL (mg min/dL)	0 (0–0)	0 (0–0)	0.65
00.00–06.00 h AOC, $<70$ mg/dL (mg min/dL)	0 (0–0)	0 (0–0)	0.32
08.00–12.00 h AOC, $<70$ mg/dL (mg min/dL)	0 (0–0)	0 (0–0)	1

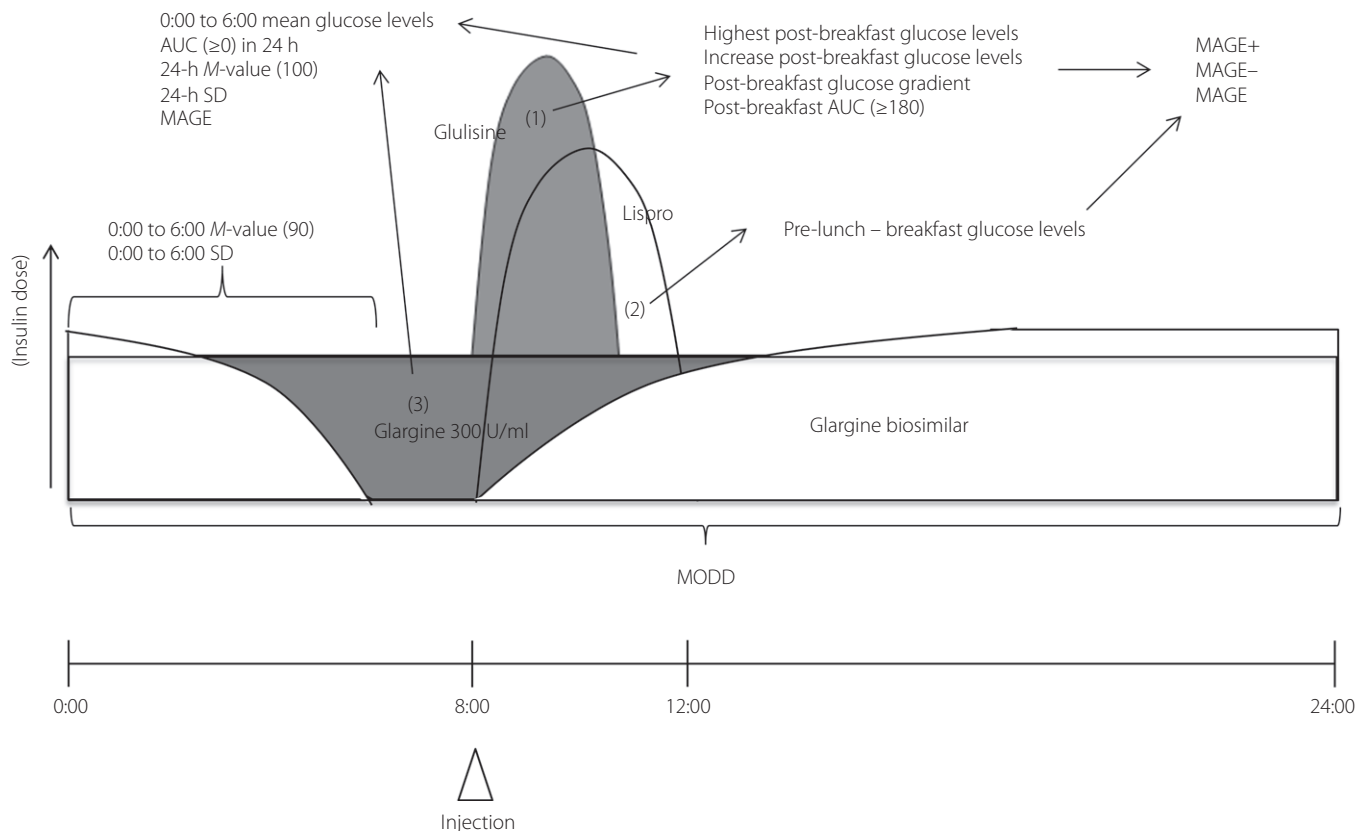
Data are shown as median (interquartile range). P, Wilcoxon signed-rank test. AOC, area over the glucose curve; AUC, area under the glucose curve; MAGE, mean amplitude of glycemic excursion.

of glulisine reduce more rapidly than those of lispro. Namely, we believe that pre-lunch glucose levels decreased too much, because lispro was affected too much by the difference of insulin concentration (part 2 of Figure 2). It has been reported that the mean residence time of glulisine was approximately half of

that of lispro or rapid-acting insulin.<sup>26</sup> The wearing off of glulisine's effect has been suggested to be faster than lispro or rapid-acting insulin, and this supports the present study results. Therefore, we believe that morning lispro administration might cause hypoglycemia before lunch, but morning glulisine



## ◆ Estimate insulin concentration



**Figure 2** | The graph shows a schematic diagram of the estimated insulin concentration in glulisine + insulin glargine 300 U/mL and lispro + insulin glargine biosimilar. AUC ( $\geq 180$ ), area under the glucose curve ( $\geq 180$  mg/dL) within 3 h; Glargine 300 U/mL, insulin glargine 300 U/mL; Glargine biosimilar, insulin glargine biosimilar; Glulisine, insulin glulisine; Highest post-breakfast glucose levels, highest post-breakfast glucose level within 3 h; Increase post-breakfast glucose levels, differences between pre-breakfast and highest post-breakfast glucose levels; Lispro, insulin lispro; MAGE+, mean amplitude of glycemic excursion of increased glucose levels; MAGE-, mean amplitude of glycemic excursion of decreased glucose levels; MAGE, mean amplitude of glycemic excursion; MODD, mean of daily difference; SD, standard deviation.

administration can avoid the risk of hypoglycemia before lunch in patients with low pre-breakfast glucose levels. We believe that the rapid onset and fast wearing off of glulisine's effect (Figure 2 [1] and Figure 2 [2]) led to the result that MAGE+, MAGE- and MAGE were significantly lower in patients taking G + G300 than those taking L + GB, because those characteristics of glulisine's action reduced rapid increases and decreases of morning glucose levels. It has been reported that the concentration of protein kinase C- $\beta$ , which is an index of oxidative stress, increases when glucose concentration decreases; that is, from hyperglycemia status to normoglycemia status.<sup>27</sup> Therefore, it is significant that glulisine can reduce not only rapid increases, but also rapid decreases of glucose levels. The result that 00.00–06.00 h M-value and 00.00–06.00 h SD were significantly lower in patients taking G + G300 than those taking L + GB was thought to be caused by the difference in serum insulin concentration between insulin glargine biosimilar (glargine BS) and glargine 300 U/mL between 00.00–06.00 h

(Figure 2). The duration of action as a clinical effect of glargine BS was thought to be approximately 22.8 h.<sup>28,29</sup> In contrast, the duration of action as a clinical effect of glargine 300 U/mL was thought to be just 24 h.<sup>30</sup> Therefore, we believe that glargine 300 U/mL improves nocturnal glycemic variability more than glargine BS, because the serum concentration of glargine BS varies, but that of glargine 300 U/mL is stable between 00.00–06.00 h (Figure 2). The result that 00.00–06.00 h mean glucose levels, AUC ( $\geq 0$ ) in 24 h, 24-h SD, 24-h M-value and MAGE were significantly lower in patients taking G + G300 than those taking L + GB was thought to be due to the fact that glargine 300 U/mL improves 24-h glycemic variability with a focus on night-time more than glargine BS<sup>30</sup> (Figure 2 [3]), and that glulisine improves 24-h glycemic variability through improvement of pre- and post-breakfast glycemic variability more than lispro,<sup>14</sup> namely, the combination therapy of glargine 300 U/mL and glulisine. The result that MODD was significantly lower in patients taking G + G300 than those taking L + GB was

thought to be caused by the small variance in daily effect of glargine 300 U/mL. Day-to-day variability (coefficient of variation %) in the area under the curve of glucose infusion rate over 24 h of glargine BS is 73%.<sup>29,31</sup> In contrast, that of glargine 300 U/mL is 34.8%.<sup>32</sup> Daily variance of glargine 300 U/mL is much smaller than that of glargine BS, and this supports the present study results. Regarding daily variance, it has been suggested that larger daily variance of glycosylated hemoglobin and glucose levels causes a higher incidence of cardiovascular events and is associated with a poor prognosis.<sup>33</sup> Daily stability of the glargine 300 U/mL effect is thought to improve cardiovascular events and prognosis.

In the present study, it was suggested that nocturnal glucose level was significantly lower in patients taking G + G300 than those taking L + GB. Decreasing nocturnal glucose levels might be interpreted as increasing the potential risk of hypoglycemia. However, at the same time, nocturnal glycemic variability was significantly lower in patients taking G + G300 than those taking L + GB in the present study. We believe that reducing glucose levels with decreasing variability does not increase the potential risk of hypoglycemia. In the present study, hypoglycemia was not significantly different between groups.

From the viewpoint of diabetes education, the practice of not eating breakfast in order to decrease post-breakfast glucose levels might exist amongst the diabetic population. However, it has been reported that the incidence of diabetes is significantly increased in people who miss breakfast than in those who eat breakfast.<sup>34</sup> Missing breakfast is thought to cause worsening of glycemic control. In a clinical trial that provides detailed support for this theory, it has been reported that post-lunch and supper glucose levels increase more in patients missing breakfast than in those eating breakfast.<sup>35</sup> In addition, the second meal effect that increased post-lunch glucose levels was lower than the increased post-breakfast glucose levels that have been reported.<sup>11</sup> Post-lunch glucose levels increase more in patients skipping breakfast than those taking breakfast. High serum concentration of free fatty acids reduces insulin sensitivity. Free fatty acids levels are reduced to a greater extent before lunch than before breakfast by eating breakfast, and this causes the second meal effect. Post-lunch glucose levels increase in patients missing breakfast, because free fatty acids levels do not decrease, and thus insulin sensitivity does not improve. Therefore, missing breakfast causes an increase in post-lunch and supper glucose levels, and worsening of glycemic control. Patients should eat breakfast, and post-breakfast glucose levels should be reduced by medication.

An advantage of the combination therapy of glargine 300 U/mL and glulisine considered from the viewpoint of diabetes education is that the injection is only given in the morning. In this regard, this combination therapy is almost the same as basal insulin therapy, and significant hypoglycemic actions can be expected as a result of adding glulisine. Another combination therapy consisting of basal

insulin + glucagon-like peptide-1 receptor agonist (GLP-1 RA) exists. However, GLP-1 RA is costly, and its digestive side-effects should be considered. Furthermore, goals of treatment are different between insulin and GLP-1 RA. Therefore, combination therapy of basal insulin and GLP-1 RA should be considered as being fundamentally different from combination therapy of morning long-acting insulin and ultra-rapid-acting insulin. Combination long-acting and ultra-rapid-acting insulin given as a single morning injection might be more desirable to patients, as it is easier to build into a daily routine, in a similar way to the habit of teeth-brushing, for example, and is thus less likely to be forgotten or delayed. Understandably, patients might not enjoy injecting insulin, and so administering this necessary treatment at the start of the day could feasibly improve the quality of life for the diabetes patient. Additionally, given the importance of eating breakfast in the diabetic population, morning administration of insulin might help those who would previously have missed breakfast to remember to eat at this time, by linking the treatment with eating as part of the patient's daily routine.

In the present study, it was suggested that the combination therapy of glulisine + glargine 300 U/mL decreases post-breakfast glucose level reducing rate of rise of that, nocturnal and 24-h glucose variability and levels without causing hypoglycemia, and daily variance. As an insulin therapy that is injected in the morning only, the combination therapy of glulisine + glargine 300 U/mL is suggested to be the best therapy not only to improve glycemic variability, but also from a patient preference viewpoint. Thus, the clinical significance of the present study is high. However, this study had limitations as a single facility, open-label study. We shall endeavor to address these limitations by gathering more cases and carrying out further clinical studies in the future.

## ACKNOWLEDGMENTS

This work was supported in part by the General Inuyamachuo Hospital.

## DISCLOSURE

The authors declare no conflict of interest.

## REFERENCES

1. Tsujimoto T, Yamamoto-Honda R, Kajio H, *et al.* Vital signs, QT prolongation, and newly diagnosed cardiovascular disease during severe hypoglycemia in type 1 and type 2 diabetic patients. *Diabetes Care* 2014; 37: 217–225.
2. Bolli GB, Perriello G, Fanelli CG, *et al.* Nocturnal blood glucose control in type I diabetes mellitus. *Diabetes Care* 1993; 16: 71–89.
3. Currie JC, Peters RJ, Tynan A, *et al.* Survival as a function of HbA1c in people with type 2 diabetes: a retrospective cohort study. *Lancet* 2010; 375: 481–489.
4. Mendez CE, Mok KT, Ata A, *et al.* Increased glycemic variability is independently associated with length of stay

- and mortality in noncritically ill hospitalized patients. *Diabetes Care* 2013; 36: 4091–4097.
5. Takeishi S, Mori A, Hachiya H, *et al.* Hypoglycemia and glycemic variability are associated with mortality in non-intensive care unit hospitalized infectious disease patients with diabetes mellitus. *J Diabetes Investig* 2016; 7: 429–435.
  6. Takeishi S, Mori A, Kawai M, *et al.* Major Increases between Pre- and Post-breakfast Glucose Levels May Predict Nocturnal Hypoglycemia in Type 2 Diabetes. *Intern Med* 2016; 55: 2933–2938.
  7. Tanaka Y, Atsumi Y, Asahina T, *et al.* Usefulness of revised fasting plasma glucose criterion and characteristics of the insulin response to an oral glucose load in newly diagnosed Japanese diabetic subjects. *Diabetes Care* 1998; 21: 1133–1137.
  8. Guideline of American Diabetes Association. Standards of medical care in diabetes–2015: summary of revisions. *Diabetes Care* 2015; 38: S4.
  9. Terauchi Y, Koyama M, Cheng X, *et al.* New insulin glargine 300 U/mL versus glargine 100 U/mL in Japanese people with type 2 diabetes using basal insulin and oral antihyperglycaemic drugs: glucose control and hypoglycaemia in a randomized controlled trial (EDITION JP 2). *Diabetes Obes Metab* 2016; 18: 366–374.
  10. Ando K, Nishimura R, Tsujino D, *et al.* 24-hour glycemic variations in drug-naïve patients with type 2 diabetes: a continuous glucose monitoring (CGM)-based study. *PLoS One* 2013; 8: e71102.
  11. Jovanovic A, Gerrard J, Taylor R. The second-meal phenomenon in type 2 diabetes. *Diabetes Care* 2009; 32: 1199–1201.
  12. Monnier L, Colette C, Sardinoux M, *et al.* Frequency and severity of the dawn phenomenon in type 2 diabetes: relationship to age. *Diabetes Care* 2012; 35: 2597–2599.
  13. Takeishi S, Mori A, Kawai M, *et al.* Investigating the relationship between morning glycemic variability and patient characteristics using continuous glucose monitoring data in patients with Type 2 Diabetes. *Intern Med* 2016 (Accepted).
  14. Urakami T, Kuwabara R, Habu M, *et al.* Efficacy and safety of switching to insulin glulisine from other rapid-acting insulin analogs in children with type 1 diabetes. *J Diabetes Investig* 2015; 6: 87–90.
  15. Morishita N. Efficacy and safety of insulin glulisine in patients with insufficiently controlled Type1 and Type2 diabetes. *J New Rem & Clin* 2013; 62: 73–79.
  16. Mori Y, Ohta T, Shiozaki M, *et al.* The effect of a low-carbohydrate/high-monounsaturated fatty acid liquid diet and an isoleucine-containing liquid diet on 24-h glycemic variability in diabetes patients on tube feeding: a comparison by continuous glucose monitoring. *Diabetes Technol Ther* 2012; 14: 619–623.
  17. Service FJ, Molnar GD, Rosevear JW, *et al.* Mean amplitude of glycemic excursion, a measure of diabetic instability. *Diabetes* 1970; 19: 644–655.
  18. Service FJ, Nelson RL. Characteristics of glycemic stability. *Diabetes Care* 1980; 3: 58–62.
  19. Kovatchev BP, Otto E, Cox D, *et al.* Evaluation of a new measure of blood glucose variability in diabetes. *Diabetes Care* 2006; 29: 2433–2438.
  20. Nishimura R, Tsujino D, Taki K, *et al.* Continuous glucose monitoring with Humalog Mix 25 versus Humalog Mix 50, twice daily: a comparative pilot study -results from the Jikei-EVALUATION of insulin Lispro mixture on pharmacodynamics and glycemic Variance (J-EVOLVE) study. *Cardiovasc Diabetol* 2010; 9: 16.
  21. Schlichtkrull J, Munck O, Jersild M. The M-value, an index of blood-sugar control in diabetics. *Acta Med Scand* 1965; 177: 95–102.
  22. Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group. Effectiveness of continuous glucose monitoring in a clinical care environment: evidence from the Juvenile Diabetes Research Foundation Continuous Glucose Monitoring (JDRF-CGM) trial. *Diabetes Care* 2010; 33: 17–22.
  23. Tanaka N, Hiura Y. Effects of rapid-acting insulin analogues insulin glulisine and insulin aspart on postprandial glycemic excursion with single bout of exercise in patients with type 2 diabetes. *Endocrine J* 2015; 62: 411–416.
  24. Sakamoto M, Nishimura R, Irako T, *et al.* Comparison of vildagliptin twice daily vs. sitagliptin once daily using continuous glucose monitoring (CGM): crossover pilot study (J-VICTORIA study). *Cardiovasc Diabetol* 2012; 11: 92.
  25. Morrow L, Muchmore DB, Hompesch M, *et al.* Comparative pharmacokinetics and insulin action for three rapid-acting insulin analogs injected subcutaneously with and without hyaluronidase. *Diabetes Care* 2013; 36: 273–275.
  26. Common Technical Document, 2. Clinical Overview, Apidra, Sanofi KK.
  27. Quagliaro L, Piconi L, Assaloni R, *et al.* Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cells: the role of protein kinase C and NAD(P)H-oxidase activation. *Diabetes* 2003; 52: 2795–2804.
  28. Poon K, King AB. Glargine and detemir: safety and efficacy profiles of the long-acting basal insulin analogs. *Drug Healthc Patient Saf* 2010; 2: 213–223.
  29. Linnebjerg H, Lam EC, Seger ME, *et al.* Comparison of the pharmacokinetics and pharmacodynamics of LY2963016 insulin glargine and EU- and US-approved versions of Lantus insulin glargine in healthy subjects: three randomized euglycemic clamp studies. *Diabetes Care* 2015; 38: 2226–2233.
  30. Monnier L, Owens DR, Bolli GB. The new long-acting insulin glargine U300 achieves an early steady state with low risk of accumulation. *Diabetes Metab* 2016; 42: 77–79.



31. Heise T, Hermanski L, Nosek L, *et al.* Insulin degludec: four times lower pharmacodynamic variability than insulin glargine under steady-state conditions in type 1 diabetes. *Diabetes Obes Metab* 2012; 14: 859–864.
32. Becker RH, Nowotny I, Teichert L, *et al.* Low within- and between-day variability in exposure to new insulin glargine 300 U/mL. *Diabetes Obes Metab* 2015; 7: 261–267.
33. Hirakawa Y, Arima H, Zoungas S, *et al.* Impact of visit-to-visit glycemic variability on the risks of macrovascular and microvascular events and all-cause mortality in type 2 diabetes: the ADVANCE trial. *Diabetes Care* 2014; 37: 2359–2365.
34. Uemura M, Yatsuya H, Hilawe EH, *et al.* Breakfast skipping is positively associated with incidence of type 2 diabetes mellitus: evidence from the Aichi Workers' Cohort Study. *J Epidemiol* 2015; 25: 351–358.
35. Jakubowicz D, Wainstein J, Ahren B, *et al.* Fasting until noon triggers increased postprandial hyperglycemia and impaired insulin response after lunch and dinner in individuals with type 2 diabetes: a randomized clinical trial. *Diabetes Care* 2015; 38: 1820–1826.