Comparison of daily glucose excursion by continuous glucose monitoring between type 2 diabetic patients receiving biphasic insulin aspart 30 or biphasic human insulin 30

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ABSTRACT

Aims/Introduction: Biphasic insulin aspart 30 (BIAsp 30) has an earlier and stronger peak effect with a similar duration of action to biphasic human insulin 30 (BHI 30). However, direct comparison of daily glucose excursion during treatment with these two types of insulin has not been carried out.

Materials and Methods: We carried out continuous glucose monitoring (CGM) and evaluated the 48-h glucose profile during twice-daily injections of BIAsp 30 or BHI 30 at the same dosage in 12 hospitalized patients with type 2 diabetes who participated in a randomized cross-over trial.

Results: The 48-h average glucose level and mean amplitude of glucose excursion (MAGE) were lower during BIAsp 30 treatment than with BHI 30. The average glucose level during 2–3 h after breakfast and 2–4 h after dinner, and the incremental postprandial glucose from just before to 4 h after dinner were lower with BIAsp 30 treatment than with BHI 30. Furthermore, BIAsp 30 treatment reduced the SD from 30 min before to 4 h after breakfast and lunch compared with BHI 30. The average glucose level and SD during the 30 min before each meal and during the night were not different between the two insulin preparations, and hypoglycemia was not observed with either treatment.

Conclusions: Twice-daily BIAsp 30 reduced the 48-h average glucose and MAGE, the postprandial glucose (after breakfast and dinner), and the SD of glucose excursion (after breakfast and lunch) compared with the same dosage of BHI 30, without causing hypoglycemia or deterioration of glycemic control before meals and at night. This trial was registered with UMIN (no. UMIN000005129).

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KEY WORDS: Biphasic insulin aspart 30, Biphasic human insulin 30, Continuous glucose monitoring

INTRODUCTION

Insulin aspart is a human insulin analog that is designed for rapid absorption after subcutaneous injection, and has a faster onset and shorter duration of action compared with regular human insulin as a result of substitution of aspartic acid for proline at position B28 in the B chain of the insulin molecule[1]. Although regular human insulin needs to be injected approximately 30 min before meals because of delayed absorption, insulin aspart more closely mimics the physiological postprandial insulin response and patients can inject it immediately before meals. Biphasic insulin aspart 30 (BIAsp 30) is a premixed insulin formulation that contains 30% soluble insulin aspart and 70% protamine-bound insulin aspart. Compared with biphasic human insulin 30 (BHI 30), which consists of 30% regular human insulin and 70% neutral protamine Hagedorn (NPH) insulin, BIAsp 30 shows earlier and stronger peak activity with a similar duration of action[1]. It was previously shown that the detection rate of low glucose levels (<3.5 mmol/L [63 mg/dL]) by continuous glucose monitoring (CGM) and the frequency of self-reported episodes of hypoglycemia were lower during twice-daily treatment with BIAsp 30 than during BHI30 therapy by a double-blind cross-over trial in patients with type 2 diabetes[2]. Recently, a lower risk of nocturnal hypoglycemia in type 2 diabetic patients receiving twice-daily BIAsp 30 compared with BHI was shown by a meta-analysis of nine previous trials[3]. However, insulin aspart is more rapidly absorbed after being cleaved from protamine, and it remains unclear whether or not the duration of action and potency of protamine-bound insulin aspart are similar to those of NPH insulin. Furthermore, a direct comparison of postprandial glucose excursion between BIAsp 30

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and BHI 30 has not been carried out by CGM. Thus, to investigate the influence of twice-daily BIAsp 30 on glycemic control, we compared the 48-h glucose profile between twice-daily treatment with BIAsp 30 or BHI 30 by CGM in an open-label cross-over trial of hospitalized type 2 diabetic patients with a standard daily schedule and diet.

MATERIALS AND METHODS

Patients

A total of 12 Japanese patients with type 2 diabetes (10 men and two women, aged 59.5 ± 13.1 years) (mean ± SD) were studied. The patients were recruited from the outpatient clinic of St. Marianna University Hospital (Kanagawa, Japan). Inclusion criteria were stable, but inadequate, glycemic control (HbA_{1c} > 7.8% and variation of HbA_{1c} by <0.5% within 3 months before enrolment) and treatment with a sulfonylurea only (not insulin with or without other oral anti-diabetic agents). The exclusion criteria included pregnancy, severe medical illnesses, anemia, renal failure (serum creatinine > 2.0 mg/dL), overt proteinuria, chronic liver disease, thyroid disease, malignancy or severe hypoglycemia requiring assistance within the previous 6 months. All patients gave written informed consent and the study was approved by the Ethics Committee of St. Marianna University School of Medicine (No. 1305).

HbA_{1c} (%) was estimated as the National Glycohemoglobin Standardization Program (NGSP) equivalent value, which was calculated as HbA_{1c} (NESP) (%) = HbA_{1c} (JDS) (%) + 0.4%, considering the relationship of HbA_{1c} (NGSP) values to HbA_{1c} (JDS) (%) values measured by the Japanese standard and measurement method[4].

Cross-Over Treatment With BHAsp 30 and BHI 30

After enrolment, patients were randomized to the BIAsp 30 group or BHI 30 group. Then, sulfonylurea therapy was suspended and insulin was started twice daily (before breakfast and dinner) from a dose of 0.3 U/kg per day at the outpatient clinic. BIAsp 30 and BHI 30 were injected just before meals and 30 min before meals, respectively. The insulin dosage was adjusted to achieve individual target levels, which were set by the attending physician considering each patient's clinical condition. After the insulin dosage had been fixed, the patients were admitted to St. Marianna University Hospital for the cross-over study of BIAsp 30 and BHI 30. At least 7 days after admission, a CGM device (Medtronic MiniMed, Northridge, CA, USA) was attached for 72 consecutive hours while the patient remained on the same insulin dosage. During the CGM study, the patients used a blood glucose self-monitoring device (One Touch Ultra; Life scan, Milpitas, CA, USA) and input the data into the CGM recorder for calibration at least four times daily. After the CGM study was finished, the insulin preparation was switched from BHIAsp 30 to BHI 30 or vice versa without a change of dosage. From the day after switching of insulin, a second CGM study was carried out for 72 h in the same way.

Assessment of CGM Parameters and Data Analysis

After downloading the recorded data, the following parameters were analyzed from the intermediate 48 h of data: average glucose level (AG), SD of glucose, mean amplitude of glucose excursion (MAGE), area under the glucose curve (AUC-glu) during the 30-min period before each meal and at 1–2, 2–3 and 3–4 h after each meal, and during the night (22.00–07.00 hours), and area under the curve of incremental (baseline-corrected) postprandial glucose from just before to 4 h after each meal ($IAUC_{0-4\ h}$). MAGE was calculated by taking the arithmetic mean of glucose increase and descending segments exceeded the value of 1 SD[5]. Data are presented as the mean ± SD. The two-tailed unpaired Student's *t*-test was used for statistical analysis of differences of mean values between the groups and a *P*-value of <0.05 was accepted as showing statistical significance.

RESULTS

Baseline characteristics of the patients on admission are listed in Table 1. As shown in Figure 1, the 48-h average glucose level and MAGE were significantly lower with BIAsp 30 treatment than with BHI 30, despite the same insulin dosage (142.8 ± 33.2 vs 154.8 ± 44.5 mg/dL, 93.0 ± 49.7 vs 108.0 ± 51.9 mg/dL, $P < 0.05$), but the SD did not differ between the two insulin preparations. Comparison of AUC-glu and SD from 30 min before to 4 h after each meal is shown in Table 2. From 30 min before to 4 h after breakfast and dinner, AUC-glu was significantly lower during BIAsp 30 treatment than with BHI 30, and the SD from 30 min before to 4 h after breakfast and lunch was significantly lower with BIAsp 30 than BHI 30. As shown in Table 3, SD during the 30 min before each meal and during the night did not differ between the two insulin preparations. Comparison of segmental average glucose levels (during the 30 min before each meal, as well as 1–2, 2–3 and 3–4 h after each meal, and during the night [22.00–07.00 hours]) is shown in Figure 2. The average glucose level during 2–3 h after breakfast, and during 2–3 and 3–4 h after dinner were significantly lower with

Table 1 | Patient characteristics

Sex (male:female)	12 (10:2)
Age (years)	59.5 ± 13.1
BMI (kg/m^2)	22.1 ± 2.7
Duration of diabetes (years)	8.7 ± 10.0
HbA_{1c} (JDS) (%)	8.4 ± 1.6
Diabetic complications	
Retinopathy	3
Nephropathy	3
Neuropathy	3

Data are expressed as the mean ± SD or number. HbA1c: The value of HbA1c (%) was estimated as the NGSP equivalent value (%), which was calculated as HbA1c (NGSP) (%) = HbA1c (JDS) (%) + 0.4%, considering the relation of HbA1c (JDS) (%) measured with the Japanese standard substance and measurement method to HbA1c (NGSP).
BMI, body mass index.

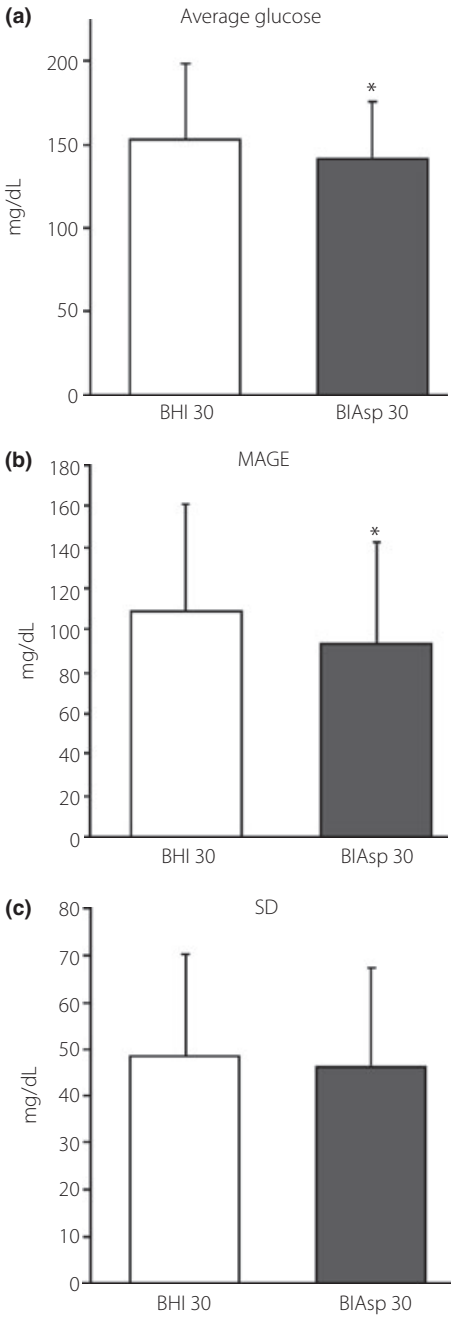

Figure 1 | Comparison of 48-h (a) average glucose, (b) mean amplitude of glucose excursion (MAGE) and (c) SD between biphasic human insulin 30 (BHI 30) and biphasic insulin aspart 30 (BIAsp 30). Data are expressed as the mean ± SD, $n = 12$. *Significant at $P < 0.05$.

BIAsp 30 treatment than with BHI 30 (182.6 ± 65.2 vs 198.6 ± 77.7 mg/dL, 155.4 ± 41.3 vs 184.6 ± 60.2 mg/dL, 137.7 ± 30.7 vs 158.6 ± 49.2 mg/dL, $P < 0.05$). Furthermore, $IAUC_{0-4\ h}$ after dinner was significantly lower with BIAsp 30 treatment than with BHI 30, as shown in Table 4. Hypoglycemia with a glucose level <70 mg/dL was not observed during treatment with both insulin preparations.

Table 2 | Area under the curve for average glucose and standard deviations from 30 min before to 4 h after each meal

Meal	Insulin preparation	AUC average glucose (mg/dL)	*P*-value	SD (mg/dL)	*P*-value
Breakfast	BHI 30	850.9 ± 263.7	<0.05	27.2 ± 9.4	<0.01
	BIAsp 30	790.4 ± 231.8		19.6 ± 9.0	
Lunch	BHI 30	924.3 ± 336.7	0.75	29.4 ± 10.9	<0.05
	BIAsp 30	905.0 ± 281.1		21.2 ± 9.1	
Dinner	BHI 30	924.3 ± 336.7	<0.05	27.6 ± 15.1	0.25
	BIAsp 30	780.7 ± 171.0		22.8 ± 8.9	

Data are expressed as the mean ± SD. Breakfast is measured from 30 min before breakfast to 4 h after breakfast. Lunch is measured from 30 min before lunch to 4 h after lunch. Dinner is measured from 30 min before dinner to 4 h after dinner.
AUC, area under the curve; BHI 30, biphasic human insulin 30; BIAsp 30, biphasic insulin aspart 30.

Table 3 | Standard deviation during the 30 min before each meal and during the night

	Insulin preparation	SD (mg/dL)	*P*-value
Before breakfast	BHI 30	12.5 ± 8.6	0.22
	BIAsp 30	8.1 ± 7.3	
Before lunch	BHI 30	14.3 ± 10.4	0.25
	BIAsp 30	11.6 ± 8.4	
Before dinner	BHI 30	21.2 ± 19.1	0.18
	BIAsp 30	14.2 ± 12.8	
Night	BHI 30	21.3 ± 9.4	0.22
	BIAsp 30	17.6 ± 8.3	

Data are expressed as the mean ± SD.
BHI 30, biphasic human insulin 30; BIAsp 30, biphasic insulin aspart 30.

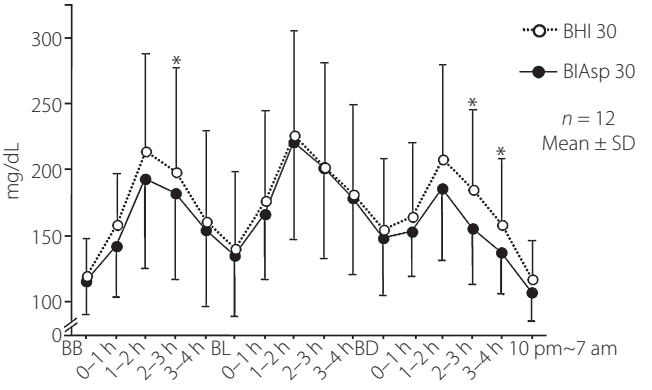

Figure 2 | Average glucose profile during biphasic human insulin 30 (BHI 30) or biphasic insulin aspart 30 (BIAsp 30) treatment. BB, before breakfast (−0.5 to 0 h); BD, before dinner (−0.5 to 0 h); BL, before lunch (−0.5 to 0 h). *Significant at $P < 0.05$.

Table 4 | Postprandial mean incremental area under the curve for glucose

	Insulin preparation	SD (mg/dL h)	*P*-value
$IAUC_{0-4\ h}$ (breakfast)	BHI 30	152.9 ± 56.8	0.08
	BIAsp 30	139.5 ± 48.2	
$IAUC_{0-4\ h}$ (lunch)	BHI 30	161.2 ± 60.1	0.69
	BIAsp 30	158.5 ± 52.1	
$IAUC_{0-4\ h}$ (dinner)	BHI 30	140.2 ± 46.4	<0.05
	BIAsp 30	121.1 ± 32.3	

Data are expressed as the mean ± SD. $IAUC_{0-4\ h}$ measured from 0 to 4 h after each meal.
AUC, area under the curve; BHI 30, biphasic human insulin 30; BIAsp 30, biphasic insulin aspart 30; IAUC, incremental area under the curve for glucose.

DISCUSSION

The present study showed that treatment with BIAsp 30 improved the 48-h average glucose level and MAGE compared with the same dosage of BHI 30, whereas the SD of glucose excursion did not differ between the two insulin preparations. In addition, the glucose level from 30 min before to 4 h after breakfast and dinner, especially the average glucose level during 2–3 h after breakfast and during 2–4 h after dinner, and baseline-corrected incremental postprandial glucose from just before to 4 h after dinner were lower with BIAsp 30 treatment than with BHI 30. Furthermore, BIAsp 30 reduced the SD from 30 min to 4 h after breakfast and lunch compared with BHI30, although the 48-h SD was not different. Finally, hypoglycemic episodes were not observed, and the average glucose level and SD during the night did not differ between the two insulin preparations.

Previously, McSorley *et al.*[6] compared the pharmacokinetics and pharmacodynamics of BIAsp 30 (twice daily before breakfast and dinner) with the equivalent dose of BHI30 in a double-blind cross-over study of type 2 diabetic patients. They assessed the 24-h serum insulin and glucose profiles by obtaining blood samples just before each meal and then at 15-min intervals for 2 h, half-hourly for 1 h, and hourly until the next meal. They observed that the time to the maximum serum insulin concentration (T_{max}) after the morning and evening injections was significantly shorter with BIAsp 30 than BHI 30 (94 ± 35 vs 155 ± 42, 89 ± 32 vs 137 ± 83 min, mean ± SD), whereas the maximum serum insulin concentration (C_{max}) after breakfast and dinner was significantly higher with BIAsp 30 than BHI 30 (108 ± 55 vs 81 ± 45, 96 ± 54 vs 79 ± 43 mU/L). Thus, the area under the concentration vs time curve of insulin during the 2 h after insulin injection for breakfast and dinner was larger with BIAsp 30 than BHI 30 (144 ± 68 vs 102 ± 55, 136 ± 72 vs 114 ± 66 mU/L per hour). According to these results, early postprandial glucose levels within 2 h after breakfast and dinner are expected to be lower with BIAsp 30 than with BHI 30. Indeed, we found that glucose excursion during the 4 h after breakfast and dinner was smaller with BIAsp 30 than BHI 30, but a difference in the early postprandial period (1–2 h) after breakfast or dinner was not shown. As can be seen in Figure 2, the present study showed that a significant difference of glucose was not observed within 2 h postprandially, but was noted in the late phase (2–3 h after breakfast and 2–4 h after dinner). These results suggest that the improvement of postprandial glucose by BIAsp 30 might occur later than its T_{max}. Thus, we should be careful about the possibility of hypoglycemia, even in the late postprandial period (2–4 h after breakfast or dinner) when switching from BHI 30 to BIAsp 30. Also, for further improvement of early postprandial glucose, it might be useful to add an α-glucosydase inhibitor (α-GI), such as miglitol, which was reported to reduce postprandial glucose more markedly at 1 h after meals than other α-GI agents[7–9].

Recent studies have shown that postprandial hyperglycemia or fluctuation of the glucose profile might be important risk factors for macrovascular complications independent of HbA_{1c} in diabetic patients[10–18]. Chen *et al.*[19] reported a significant correlation between glucose fluctuation by CGM analysis and the carotid intima-media thickness (IMT) in type 2 diabetic patients. We have already reported that the levels of 1,5-anhidroglucitol (1,5-AG) and glycated albumin (GA), but not HbA_{1c}, were correlated with both the average glucose level and the SD by CGM analysis of type 2 diabetic patients[20]. Similarly, Dungan *et al.*[21] observed that 1,5-AG not only reflected the average glucose level, but also glucose fluctuation, on CGM analysis of type 1 and type 2 diabetic patients. Interestingly, Ohira *et al.*[22] evaluated the effect of switching from BHI 30 (twice daily) to the same dose of BIAsp 30 on arterial stiffness measured by the cardio-ankle vascular index (CAVI) in type 2 diabetic patients. They found a significant negative correlation between the change of CAVI and the change of 1,5-AG, but not that of HbA_{1c}, at 3 months after switching. This suggests an association of the improvement of arterial stiffness with improvement of postprandial hyperglycemia and glucose fluctuation by switching from BHI 30 to BIAsp 30. Consistent with the previous reports[2,6,22], we confirmed amelioration of postprandial glucose by BIAsp 30 treatment. Furthermore, we first showed improvement of glucose fluctuation (the 48-h MAGE and the SD values from 30 min before to 4 h after breakfast and lunch) with BIAsp 30 measured by CGM in the present study. Taken together, it is possible that BIAsp 30 might be useful for prevention of macrovascular complications by improvement of the postprandial average glucose level and its fluctuation, and so, measurement of 1,5-AG or GA in addition to HbA_{1c} might be necessary to evaluate the effect of switching from BHI 30 to BIAsp 30. However, the number of our patients was small, so further large-sized studies are required to confirm the beneficial effect of BIAsp 30.

Considering pharmacokinetics, the glucose levels before breakfast or dinner and during the night might not be

influenced by the soluble insulin aspart or regular human insulin component (30%) when BIAsp 30 or BHI 30 is injected before breakfast and dinner, but rather by the protamine-bound insulin aspart or NPH insulin component (70%). Roch *et al.* previously found no difference of C_{max} and T_{max} between protamine-bound insulin lyspro (NPL) and NPH, and observed a slightly earlier onset of action for NPL and similar duration of action by the glucose clamp technique in healthy non-diabetic subjects[23]. Although a similar comparison between protamine-bound insulin aspart and NPH has not been reported, considering the structural similarity of insulin aspart and insulin lyspro, prolonged release of insulin aspart from protamine-bound aspart might occur in a manner similar to the release of human insulin from NPH. Actually, the average glucose levels during the 30 min before each meal, after lunch and at night did not differ between the two insulin preparations in the present study, suggesting that protamine-bound insulin aspart in BIAsp 30 might have a similar effect on glycemic control at these times to NPH in BHI 30.

In conclusion, twice-daily BIAsp 30 reduced the 48-h average glucose level and MAGE, postprandial glucose after breakfast and dinner, and SD of glucose excursion after breakfast and lunch compared with the same dosage of BHI 30 without causing hypoglycemia or deterioration of glycemic control before meals and at night.

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