ORIGINAL ARTICLE

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Multiple mealtime administration of biphasic insulin aspart 30 versus traditional basal-bolus human insulin treatment in patients with type 1 diabetes

J.-W. Chen,[1,2] T. Lauritzen,[2] A. Bojesen[1] and J. S. Christiansen[1]

[1]Department of Endocrinology and Diabetes, Aarhus University Hospital, Aarhus, Denmark
[2]Department of General Practice, Aarhus University, Aarhus, Denmark

Aim: The aim of this study was to compare the effect of multiple mealtime injections of biphasic insulin aspart 30 (30% fast-acting insulin aspart in the formulation, BIAsp30) to traditional basal-bolus human insulin regimen (HI) on glycaemic control in patients with type 1 diabetes.
Methods: Twenty-three patients (eight women and 15 men) aged 44.8 (20.6–62.5) years (median and range) with a diabetes duration of 19.5 (1.6–44.6) years completed the study. All eligible patients were randomly assigned to BIAsp30 thrice daily supplied with bedtime NPH insulin when necessary, or basal-bolus HI for 12 weeks and then switched to the alternative regimen for another 12 weeks. The insulin dose adjustments were made by patients on the basis of advice from a diabetes nurse. At end of each treatment period, the patients attended two profile days, 1 week apart for pharmacodynamic and pharmacokinetic assessments. HbA_{1C} was measured at baseline and at the end of each treatment period. A seven-point self-monitored blood glucose (SMBG) was obtained twice weekly.
Results: In comparison with HI, multiple mealtime injections of BIAsp30 resulted in a significant reduction in HbA_{1C} [HI vs. BIAsp30 (%, geometric mean and range): 8.6 (7.4–11.4) vs. 8.3 (6.7–9.8), $p = 0.013$]. During treatment with BIAsp30, nighttime glycaemic control was significantly improved. Day-to-day variation in pharmacodynamics and pharmacokinetics and the rate of hypoglycaemia were not increased with BIAsp30 compared with HI.
Conclusions: In type 1 diabetics, multiple mealtime administration of BIAsp30 compared with traditional basal-bolus human insulin treatment significantly improves long-term glycaemic control without increasing the risk of hypoglycaemia. Despite a higher proportion of intermediate-acting insulin, thrice-daily injections with BIAsp30 do not increase the day-to-day variations in insulin pharmacokinetics and pharmacodynamics.
Keywords: biphasic insulin aspart, pharmacodynamics, pharmacokinetics, type 1 diabetes
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Introduction

Multiple mealtime administration of short-acting human insulin in combination with one or two injections of intermediate-acting insulin has for two decades constituted the standard approach in the quest of obtaining normoglycaemica in patients with type 1 diabetes [1–3]. However, less than 5% of patients can reach and maintain an average value of HbA_{1C} within the normal range [3]. It has been suggested that unphysiological insulin pharmacokinetics with huge day-to-day variations in absorption of intermediate-acting insulin is the

Correspondence:
Dr Jian-Wen Chen, Department of Endocrinology and Diabetes, Aarhus University Hospital, Norrebrogade 44, DK-8000, Aarhus C, Denmark.
E-mail:
wen@iekf.au.dk

major obstacle for achieving normal glycaemic control [4–6].

Mealtime administration of the fast-acting insulin analogue leads to more stable and physiological insulin profiles as compared with soluble human insulin [7,8] and consequently improves the postprandial glycaemic control [9,10]. Furthermore, long-term metabolic control as measured by HbA_{1C} has also been improved with this regimen, but only when supplied with further mealtime injections of protracted-acting insulin [11–16]. However, due to the fact that premixing fast-acting insulin analogues with human NPH insulin will result in an exchange between soluble insulin analogues and protamine-bound human insulin during long-term storage [17], the above mentioned insulin administration regimen may increase the number of daily insulin injections from four to seven and may only be accepted by patients who are well educated and highly motivated [15,16].

Biphasic insulin aspart (BIAsp) formulations are compounded of fast- (free) and intermediate-acting (protamine-crystallized) insulin aspart in various ratios. The improved pharmacokinetic and pharmacodynamic properties of fast-acting insulin aspart are well preserved in BIAsp [18,19]. It has been anticipated that multiple daily injections of intermediate-acting insulin, e.g. in the form of BIAsp, may result in more stable basal insulin levels compared with those obtained when basal insulin is administered once daily due to the fact that absorption from several smaller insulin subcutaneous depots may counterbalance each other in terms of variability [6]. So far, the clinical effect of such a regimen on long-term glycaemic control has only been tested with two formulations of biphasic insulin analogue lispro: Mix50 and Mix75 (50 and 75% fast-acting lispro in the preparation, respectively), showing no improvement in HbA_{1c} [20,21].

The aim of the present study was to compare the effect of multiple mealtime injections of biphasic insulin aspart 30 (30% fast-acting insulin aspart in the formulation, BIAsp30) to traditional basal-bolus human insulin regimen (HI) on glycaemic control and on day-to-day variation in insulin pharmacodynamics and pharmacokinetics in patients with type 1 diabetes.

Patients and Methods

The trial protocol was approved by the Danish Medicines Agency and the local ethical committee. The trial was conducted in accordance with the rule of Good Clinical Practice (GCP) and was monitored by the GCP unit of Aarhus University Hospital. Written informed consent was obtained from all patients before the trial.

Patients

Patients were included if they fulfilled the following criteria: (1) age $\geq$18 years; (2) insulin-treated type 1 diabetes diagnosed according to the ADA criteria [22]; (3) diabetes duration $\geq$12 months; (4) treated with soluble human insulin (Actrapid®) thrice daily plus bedtime NPH insulin (Insulatard®) with a total daily insulin dose $<$1.8 IU/kg; (5) BMI $<$35 kg/m^2; and mean HbA_{1c} during the last 6 months $\geq$8%. Patients were excluded if they had any diabetic complication requiring acute treatment or uncontrolled hypertension or had a history of drug and alcohol abuse or were treated with any other drug known to affect blood glucose.

Study Design

The trial was a randomised, open-labelled, two-period cross-over study.

After a 4-week run-in period, all eligible patients were symmetrically randomised either thrice-daily BIAsp30 (NovoMix®30FlexPen®, Novo Nordisk A/S, Bagsvaerd, Denmark) or three injections of soluble human (short-acting) insulin at mealtime in addition to bedtime NPH insulin (Actrapid®Pen and Insulatard®FlexPen® respectively; Novo Nordisk A/S, Bagsvaerd, Denmark) (HI) for 12 weeks, and then switched to the alternative insulin regimen for another 12 weeks. BIAsp30 was injected immediately before the meal. The injection to meal interval for administration of Actrapid® was unchanged from the patient's pretrial daily practice, which varied from 0 to 30 min before the meal. Bedtime NPH was taken at around 22:00 hours.

The initial insulin dosage was the average daily dose taken during the week before randomization and was given in a 30 : 30 : 40 ratio (breakfast : lunch : dinner) for patients who were randomised or switched to BIAsp30. For those assigned or changed to HI, the initial insulin dosage was unchanged from their pretrial treatment. During the treatment with BIAsp30, patients were advised by a diabetes nurse to take bedtime NPH if needed to control fasting hyperglycaemia. Insulin dose adjustments were made by patients themselves according to the results of self-monitored blood glucose (SMBG) and advice from a diabetes nurse. All patients were advised about the targets for good glycaemic control: fasting and preprandial blood glucose 5.0–8.0 mmol/l and postprandial blood glucose 5.0–10.0 mmol/l.

Within each treatment period, patients attended two 24-h profile days 1 week apart (at days 77 and 84 of respective period). On all profile days, patients reported to the Clinical Research Unit at 07:30 hours after an overnight fast, and then serial blood samples were taken for measuring blood glucose and insulin concentrations (the assessments of insulin pharmacodynamics and pharmacokinetics respectively). After the first sample at approximately 07:45 hours, patients administered the trial drugs following blood sampling at 08:00, 13:00 and 18:00 hours, and within 5 min thereafter were served meals. The amount and pattern of meals and snacks during profile days were as close to patients' daily life as possible and were identical on the four profile days. After each main meal, blood samples were drawn every 30 min for 2 hours, followed by hourly sampling to the next meal or 02:00 hours, and then two-hourly until the end of the profile day. On profile days, the timing for insulin injections was unchanged from patients' used daily practice as mentioned above. Furthermore, the insulin dosages remained unchanged from the day before the first profile day to the end of the respective treatment period.

During the trial, patients recorded perceived hypoglycaemic events into their diary. Major hypoglycaemic episodes were defined as symptomatic hypoglycaemia where patients were unable to handle the situation himself/herself. Minor hypoglycaemic events included symptomatic hypoglycaemia only, and those hypoglycaemic events are confirmed by blood glucose measurement $\leq$2.8 mmol/l. Hypoglycaemic events occurring during 00:00–04:00 hours were reported as nocturnal hypoglycaemia. The rate of hypoglycaemia was calculated as events/patient/week at the end of each period.

Measurements

HbA_{1C} was determined at baseline and at the end of each treatment at the local laboratory by high-performance liquid chromatography (normal range at the local laboratory: 5.1–6.2%). Daily seven-point (before and 2 h after each meal, and at bedtime) SMBG was obtained twice-weekly during the entire trial period using Glucometer® DEX® 2 (Bayer, Copenhagen, Denmark). Plasma glucose profiles were measured by the glucose oxidase method. Serum total insulin concentrations were determined after separating insulin from its antibody by acidification and gel-filtration. Measurement was performed by time-resolved immunofluorometric assay (TR-IMFA) being specific to human (PerkinElmer Life Sciences, Turku, Finland) or aspart insulin [23].

Pharmacodynamic and Pharmacokinetic Assessments

On profile days, the area under the blood glucose or total insulin concentration – time curve (AUC) was calculated according to the trapezoidal rule and was stratified into four sections ($AUC_{breakfast(0-5\ h)}$, $AUC_{lunch(0-5\ h)}$, $AUC_{dinner(0-4\ h)}$, $AUC_{dinner(4-14\ h)}$). Additional pharmacodynamic endpoints were plasma glucose concentrations before and 2 h after each meal, at bedtime and at 02:00 hours ($C_{fasting}$, $C_{2\text{-h breakfast}}$, $C_{pre\text{-lunch}}$, $C_{2\text{-h lunch}}$, $C_{pre\text{-dinner}}$, $C_{2\text{-h dinner}}$, $C_{bedtime}$ and C_{night}). The day-to-day variations in insulin pharmacodynamics and pharmacokinetics were computed as the coefficient of variation (CV), which was derived from corresponding parameters on two profile days within the respective treatment periods.

Statistical Analyses

Previous studies showed that after 12-week treatment, the difference in HbA_{1c} between multiple daily injections of different formulations of biphasic insulin analogue and basal-bolus human insulin administrations ranged from 0.1 to 0.5% [20,21]. Therefore, the present study was designed to have an 80% power to detect an absolute difference of 0.2 in HbA_{1C} between the two trial treatments with an anticipated standard deviation of 0.3, suggesting that 20 patients needed to complete the study.

After logarithmical transformation (ln), the differences in HbA_{1C}, total daily insulin dosage, SMBG, AUCs and additional pharmacodynamic endpoints were analysed by an ANOVA model with patients as random factor. The ANOVA model was adjusted for the differences in fasting blood glucose concentrations (fasting blood glucose concentrations as covariates) whenever necessary. The Wilcoxon Signed Rank test was utilized to compare the differences in day-to-day variations in pharmacodynamics and pharmacokinetics and the rate of hypoglycaemia between the two treatments. The corresponding values on the two profile days were averaged to compare the differences in insulin pharmacodynamics and pharmacokinetics between the two insulin regimens. Data were analysed using SPSS FOR WINDOWS version 11 (SPSS, Chicago, IL, USA). A p-value <0.05 was considered statistically significant.

Results

Twenty-seven type 1 diabetic patients were randomized. Four patients were withdrawn from the study, one due to personal reasons and three because of

non-compliance with the trial protocol. Twenty-three patients (eight women and 15 men) aged 44.8 (20.6–62.5) years (median and range) with a diabetes duration of 19.5 (1.6–44.6) years, whose BMI was 24.4 ± 3.0 kg/m^2 (mean ± s.d., weight 77.8 ± 10.9 kg), completed the trial. At baseline, the median (range) of total daily insulin dosage was 52 (24–106) IU/24 hours [0.64 (0.36–1.28) IU/kg].

Insulin Dosage

During treatment with BIAsp30, 11 patients decided to take bedtime NPH in addition to their thrice-daily BIAsp30 (+NPH), while the rest of patients remained on three injections of BIAsp30 (–NPH). The total daily insulin dosages during the two treatments were identical (HI vs. BIAsp30 (geometric mean and range): 50 (24–108) vs. 50 (24–106) IU/24 h and 0.65 (0.36–1.30) vs. 0.63 (0.36–1.28) IU/kg). In average, the fraction of intermediate-acting insulin was 74 (70–85)% [0.51 (0.25–0.92) IU/kg] of total daily dosage during administration with BIAsp30, while it was 38 (13–54)% [0.25 (0.05–0.58) IU/kg] during treatment with HI.

Glycaemic Control

No treatment period interaction or carry-over effect on HbA_{1C} was observed in the present study.

HbA_{1c} was significantly improved with both regimens as compared with the baseline ($p < 0.01$). The reduction in HbA_{1c} was, however, significantly greater during treatment with BIAsp30 compared with HI (table 1). Further analysis demonstrated that the difference in HbA_{1c} between the two trial treatments was mainly driven by the patients who administered bedtime NPH insulin in combination with their thrice-daily BIAsp30 (table 1).

The results of SMBG with at least four measurements per day were included into the final analyses (table 2). Both trial regimens resulted in almost similar daytime glycaemic control (from fasting to predinner). However, BIAsp30 was associated with significantly lower blood glucose concentrations at 2 h after dinner and at bedtime compared to HI ($p < 0.05$, table 2).

Pharmacodynamics and Pharmacokinetics

The day-to-day variation in insulin pharmacodynamics and pharmacokinetics ($AUC_{(0-24\ h)}$) was not significantly increased during treatment with BIAsp30 [HI vs. BIAsp30 (CV%, median and interquartile range): 11(5–19) vs. 11(7–19) for pharmacodynamics, ns; 8(3–11) vs. 6(2–21) for pharmacokinetics, ns].

Twenty-four hours blood glucose profiles are summarized in table 3. The BIAsp30 regimen was associated with 8% and about 15% reduction in overall blood glucose ($AUC_{(0-24\ h)}$) and nighttime blood glucose ($AUC_{dinner(0-4\ h)}$ and $AUC_{dinner(4-14\ h)}$), respectively, as compared to HI (table 3). Additional pharmacodynamic endpoints demonstrated that the difference in glycaemic control between two treatments was statistically significant only during the nighttime ($C_{2\text{-}h\ dinner}$, $C_{bedtime}$ and C_{night}, table 3).

There was a tendency that in patients who administered bedtime NPH during treatment with BIAsp30 (+NPH), the blood glucose excursion remained lower with BIAsp30 as compared with HI throughout the whole profile day, while it was lower only during the night in patients taking three injections of BIAsp30 (–NPH) (figure 1a,b). The statistical analyses were not performed due to reduced statistic power.

Twenty-four hours blood total insulin profiles are shown in figure 2. There was a non-significant tendency towards higher nighttime insulin concentrations during treatment with BIAsp30 as compared to that with

Table 1 HbA_{1c} at baseline and after 12 weeks treatment with multiple mealtime injections of biphasic insulin aspart 30 (BIAsp30) or traditional basal-bolus human insulin (HI) administration in patients with type 1 diabetes*

	Baseline	HI	BIAsp30
All patients	9.2 (8.1–12.3)	8.6 (7.4–11.4)†	8.3 (6.7–9.8)†‡
Patients not taking bedtime NPH insulin during treatment with BIAsp30 (–NPH, n = 12)	9.1 (8.3–10.9)	8.5 (7.5–9.8)†	8.5 (7.0–9.7)†
Patients administering bedtime NPH insulin in addition to thrice-daily BIAsp30 (+NPH, n = 11)	9.2 (8.1–12.3)	8.7 (7.4–11.4)†	8.2 (6.7–9.8)†‡

*Results are expressed as geometric mean and range.
†$p < 0.05$ as compared with baseline.
‡$p < 0.05$ BIAsp30 vs. HI.

Table 2 Geometric mean (range) of self-monitored blood glucose concentrations during the study

	HI	BIAsp30
Fasting (mmol/l)	8.5 (5.6–12.5)	8.6 (6.2–12.6)
2 h after breakfast (mmol/l)	9.1 (6.3–11.7)	9.7 (5.8–13.8)
Before lunch (mmol/l)	7.8 (5.1–12.3)	8.2 (6.0–13.4)
2 h after lunch (mmol/l)	8.4 (5.8–15.2)	8.8 (5.4–14.2)
Before dinner (mmol/l)	8.0 (5.0–15.0)	7.9 (5.0–14.0)
2 h after dinner (mmol/l)	9.6 (6.6–18.0)	8.3 (5.0–12.2)*
Bedtime (mmol/l)	9.8 (6.2–15.7)	8.2 (5.8–12.6)*

BIAsp30, biphasic insulin aspart 30; HI, human insulin.
*BIAsp30 vs. HI, $p < 0.05$.

HI [HI vs. BIAsp30 (pmol × h/l, geometric mean), ratio (BIAsp30/HI) (95% CI): 3231.6 vs. 3358.2, 1.04 (0.67–1.60) for $AUC_{dinner(0-14\ h)}$; 4905.7 vs. 5356.8, 1.09 (0.63–1.89) for $AUC_{dinner(4-14\ h)}$; (ns)].

Hypoglycaemia

The rate of hypoglycaemia was not statistically different between two regimens [HI vs. BIAsp30 (events/patient/week, median and range): 0.7 (0.0–3.3) vs. 1.2 (0.1–3.1) for total events; 0.2(0.1–0.7) vs. 0.2(0.1–0.7) for nocturnal hypoglycaemia; ns respectively). During HI, one patient had one event of major hypoglycaemia, while two patients reported a total of three major hypoglycaemic episodes during BIAsp30.

In patients who took only thrice-daily BIAsp30 (–NPH), the rate (range) of total hypoglycaemic events was 1.1 (0.3–1.9) events/patient/week, while it was 1.2 (0.1–3.1) events/patient/week in patients administrating thrice-daily BIAsp30 in addition to bedtime NPH insulin (+NPH). The statistical analyses were not performed due to reduced statistic power.

Patient Preference

At the end of the present study, 19 patients (83%) preferred continuing with multiple daily injections of BIAsp30, while four patients received basal-bolus human insulin administrations.

Discussion

The main objective of the present study was to compare the effect of multiple mealtime injections of a biphasic insulin analogue preparation to traditional basal-bolus human insulin administrations on long-term glycaemic

Table 3 Twenty-four hours blood glucose profiles during treatment with basal-bolus human insulin (HI) and multiple mealtime injections of BIAsp30*summarized as concentration (C) and area under the blood glucose curve (AUC).

			BIAsp30/HI		
	HI (mean)	BIAsp30 (mean)	Ratio	95% CI	p-value
$C_{fasting}$(mmol/l)	9.9	9.5	0.97	(0.76,1.22)	0.76
$C_{2\text{-}h\ breakfast}$(mmol/l)	13.2	12.7	0.96	(0.81,1.14)	0.64
$C_{pre\text{-}lunch}$(mmol/l)	6.6	7.2	1.10	(0.89,1.37)	0.37
$C_{2\text{-}h\ lunch}$(mmol/l)	7.1	7.4	1.05	(0.88,1.25)	0.58
$C_{pre\text{-}dinner}$(mmol/l)	8.1	7.5	0.92	(0.74,1.14)	0.43
$C_{2\text{-}h\ dinner}$(mmol/l)	9.9	7.6	0.77	(0.64,0.93)	0.01
$C_{bedtime}$ (22:00) (mmol/l)	7.4	5.8	0.79	(0.67,0.93)	0.01
C_{night} (02:00) (mmol/l)	10.3	8.5	0.82	(0.71,0.95)	0.01
$AUC_{(0-24\ h)}$† (mmol × h/l)	237.7	219.6	0.92	(0.86,0.99)	0.03
$AUC_{breakfast\ (0-5\ h)}$† (mmol × h/l)	56.8	57.5	1.01	(0.90,1.13)	0.83
$AUC_{lunch\ (0-5\ h)}$ (mmol × h/l)	39.3	39.9	1.01	(0.89,1.16)	0.82
$AUC_{dinner\ (0\text{-}4\ h)}$ (mmol × h/l)	37.1	31.0	0.84	(0.71,0.98)	0.03
$AUC_{dinner\ (4\text{-}14\ h)}$ (mmol × h/l)	103.9	88.1	0.85	(0.72,1.00)	0.05

C – blood glucose concentrations before and 2 hours after each meal, at bedtime and at 02:00 hours.
*Results are expressed as geometric means drawn from ANOVA; the mean ratio between two drugs and 95% CI for the ratio are also shown.
†The analyses are adjusted for the differences in fasting blood glucose concentrations (as covariates).

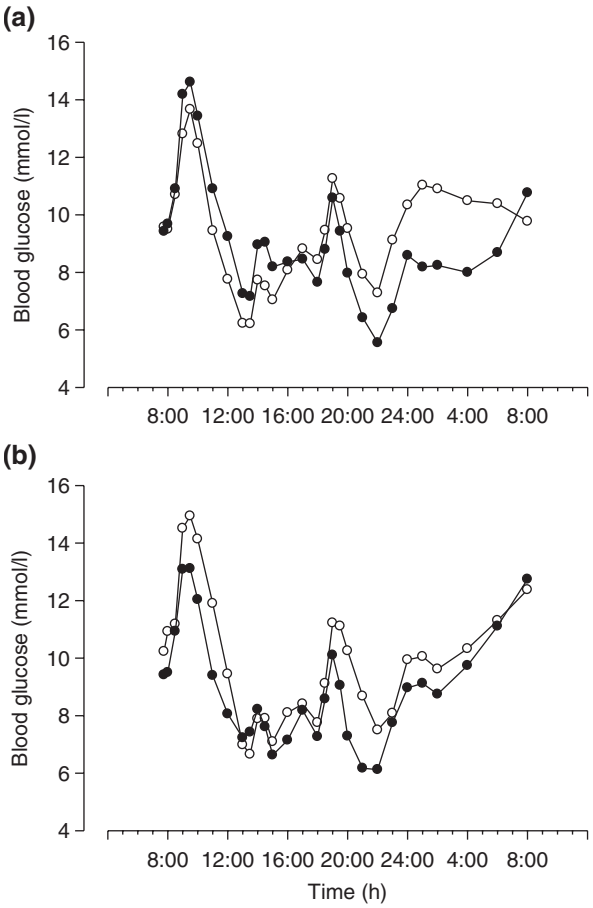

Fig. 1 Plasma glucose profiles in patients (a) taking only thrice-daily BIAsp30 (–NPH) and (b) administering thrice-daily BIAsp30 in addition to bedtime NPH insulin (+NPH). ○, human insulin preparations; ●, BIAsp30. The results are presented as geometric mean.

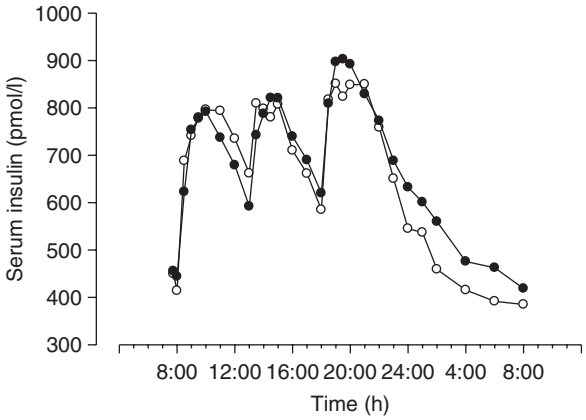

Fig. 2 Blood insulin profiles during treatment with human insulin preparations (○) or BIAsp30 (●). The results are presented as geometric mean.

control during 12-week treatment in patients with type 1 diabetes, who were poorly controlled by their pretrial regimen. Thus, one major difference between the two regimens was a doubling of the percentage of intermediate-acting insulin on a 24 h basis during BIAsp30 treatment, however, dividing the intermediate-acting insulin into three or four injections, compared with the traditional once daily injection of basal human insulin (HI).

In the present study, all patients have been suggested to take extra NPH insulin at bedtime during treatment with BIAsp30. However, the present study was not a treat-to-target trial, and patients would make their own decision on nighttime insulin administration. As the result, slightly more than 50% of patients decided not to take bedtime NPH. This may reflect the patients' fear of nocturnal hypoglycaemia – the cost of intensive glycaemic regulation [24]. It is reasonable to assume that such a risk may be overlooked by the patients in the present study, since the BIAsp30 regimen is a new manner of insulin administration, and in such a regimen, the proportion of intermediate-acting insulin is more than 70% of total daily insulin doses. But, at the end of the present study, 83% of patients would prefer continuing with multiple daily injections of BIAsp30, implying that better treatment compliance could be obtained with such an insulin regimen.

Our present study showed that in comparison to HI, the better long-term glycaemic control was achieved with BIAsp30 regimen mainly due to the improvement in nighttime blood glucose regulations, which in turn might be the consequence of higher blood insulin profiles during the night. Subgroup analysis indicated that the significant reduction in HbA_{1c} obtained with thrice-daily injections of BIAsp30 was mainly driven by the patients who also administered additional bedtime NPH insulin, of which the dosage ranged from 2 to 10 IU (3–15% of total daily insulin dosage). We further observed that in this subgroup of patients (+NPH), better nighttime glycaemic control could be extended to daytime as compared with human insulin administrations. However, during multiple mealtime injections of BIAsp30, a rapid decline in blood glucose concentrations after evening meal followed by a gradually deteriorated glycaemic control during the early morning was demonstrated even in patients taking additional bedtime NPH insulin. The finding implies that a more optimized nighttime insulin administration will be admirable to achieve an even better long-term glycaemic control as also suggested by other studies [20,21].

Nevertheless, it is reasonable to speculate that in the present study, the interpretation derived from subgroup analysis would be compromised by the reduced statistical power. Additionally, the conclusion about the differences in nighttime insulin pharmacokinetics between two trial regimens should also be taken with caution, since no statistically significant differences were observed, which might be due to a lack of statistical power. But, it is logical to assume that non-significant (4–9%) differences in insulin pharmacokinetics could result in a clinically relevant change in insulin pharmacodynamics as indicated by our recent study illustrating that relatively smaller differences in insulin profiles would result in exaggerated changes in blood glucose concentrations during the nighttime [25].

Postprandial glycaemic control in the morning was not optimal with multiple mealtime injections of BIAsp30 in the present study as indicated by the result of SMBG or profile day insulin pharmacodynamics. This may imply that the proportion of fast-acting insulin aspart (30%) in BIAsp30 is too low to sufficiently suppress the elevation in blood glucose concentration after breakfast. One of the solutions could be to exchange morning BIAsp30 with a biphasic insulin analogue formulation containing higher proportion of fast-acting insulin analogue. Therefore, it is anticipated that different formulations of biphasic insulin analogue containing various ratios of fast- and intermediate-acting insulin aspart may need to be administered at different meals to meet patient's individual requirement and that one of the advantages of such an insulin regimen may be the increased treatment compliance [15,16,26].

Earlier studies have suggested that the spontaneous intraindividual day-to-day variations in insulin absorption are huge after one or two injections of intermediate-acting insulin [4,27]. Our study demonstrated that the day-to-day variations in insulin pharmacodynamics and pharmacokinetics ($AUC_{(0-24\ h)}$) during multiple mealtime injections of BIAsp30 were 11 and 6%, respectively, which did not differ significantly from basal-bolus human insulin administrations. The day-to-day variation in insulin pharmacokinetics observed in the present study was lower than that by Heinemann and coworkers [28], who showed that after single injection of fast-acting insulin aspart on four consecutive days, the intraindividual variability was 18% for metabolic effect and 15% for insulin pharmacokinetics ($AUC_{(0-600min)}$). This might be due to the fact that the variation in insulin absorption from several smaller subcutaneous depots could counterbalance each other [4,6]. The present study indicates that more stable insulin pharmacodynamics and pharmacokinetics could be achieved with thrice-daily injections of BIAsp30, despite a higher proportion of intermediate-acting insulin (74%) being administered as compared with basal-bolus HI (38%).

Empirically, thrice-daily administrations of human biphasic insulin 30 (30% short-acting human insulin in the preparation, BHI30) may increase the frequency of hypoglycaemic episodes, especially those events occurring at a late postprandial stage due to the higher proportion of intermediate-acting insulin being given. This may partially explain why BHI30 has frequently been prescribed as a regimen with twice-daily injections [29]. Previous observations indicate that there would not be a strong tendency towards increased hypoglycaemic frequency during multiple injections of BIAsp30 [30]. Indeed, the results from the present study showed that the hypoglycaemic episodes were not significantly elevated during multiple mealtime injections of BIAsp30, which was in accordance with our previous findings [25]. These findings imply that BIAsp30 can safely be used in a thrice-daily regimen. However, it is also anticipated that various proportions of intermediate-acting insulin administered in the present study may result in different hypoglycaemic rates observed with different trial insulin regimens. Further study will be necessary to evaluate the safety of long-term treatment with multiple daily injections of BIAsp30 if tighter HbA_{1C} target is being met.

In conclusion, the regimen with thrice-daily mealtime injections of BIAsp30 is a promising alternative to classic basal-bolus insulin regimen with fast- and intermediate-acting human insulin, and one of the major advantages of such a regimen is a stable, predictable supply of basal insulin.

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