Improved Postprandial Glycemic Control With Biphasic Insulin Aspart Relative to Biphasic Insulin Lispro and Biphasic Human Insulin in Patients With Type 2 Diabetes

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OBJECTIVE — The rapid-acting insulin analogs aspart and lispro have now been developed in biphasic formulations. This trial compared the postprandial serum glucose control of biphasic insulin aspart 30 (BIAsp 30: 30% aspart, 70% protaminated aspart) with that of biphasic insulin lispro 25 (Mix25: 25% lispro, 75% protaminated lispro) and biphasic human insulin 30 (BHI 30: 30% regular insulin, 70% NPH insulin) in insulin-treated subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS — This was an open-labeled, randomized, single-dose, three-way crossover trial of 61 insulin-treated subjects with type 2 diabetes who had no significant late diabetic complications. BIAsp 30 and Mix25 were injected subcutaneously immediately before a test meal, and BHI 30 was injected 15 min before a test meal. The primary target of analysis was serum glucose excursion 0–5 h after a meal.

RESULTS — The postprandial glycemic control with BIAsp 30, as assessed by the 5-h postmeal serum glucose excursion, was superior to that with both BHI 30 and Mix25 (16.6 ± 4.5 vs. 20.1 ± 4.9 and 18.9 ± 6.1 mmol/l per hour, respectively; $P < 0.001$ and $P < 0.05$). For BIAsp 30 versus BHI 30, this was supported by a reduced maximum glucose concentration [$C_{max(SG)}$] (−5%; $P < 0.05$) occurring earlier (−13 min; $P < 0.01$). Furthermore, BIAsp 30 displayed a higher maximum serum insulin concentration (+101%; $P < 0.001$) occurring earlier (−55 min; $P < 0.001$) compared with BHI 30. Compared with Mix25, there was a shorter time to $C_{max(SG)}$ (−11 min; $P < 0.05$) after treatment with BIAsp 30.

CONCLUSIONS — This trial demonstrates that BIAsp 30 improves postprandial glycemic control compared with both Mix25 and BHI 30 in subjects with type 2 diabetes.

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Recent studies (1–5), including the Diabetes Control and Complications Trial (DCCT) (1) and the U.K. Prospective Diabetes Study (UKPDS) (4,5), have established the deleterious role of sustained hyperglycemia in the development and progression of microvascular and macrovascular complications in patients with diabetes. Furthermore, there is increasing evidence that prolonged postprandial hyperglycemia makes a major contribution to overall glucose control as well as to the development of late diabetic complications (6–8). Interestingly, analysis of the DECODE (Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe) database, which contains data from >180,000 person-years of accumulated follow-up in populations from various parts of Europe, shows that a high 2-h glucose response to an oral glucose tolerance test is associated with an increased risk of death, independent of fasting blood glucose levels (9,10). Also, the Diabetes Intervention Study (DIS) showed a significant association between postprandial glucose levels and the incidence of myocardial infarction and death rates (11). These findings underline the importance of developing treatments that most effectively improve glycemic control, including suppression of postprandial blood glucose excursions.

Current mealtime treatments with human insulin are not optimal. Rapid-acting insulin analogs such as insulin aspart have been developed to overcome the shortcomings of conventional therapies with human insulin. Insulin aspart (IAsp) is an analog of human insulin in which the amino acid proline, at position B28 on the insulin molecule, has been replaced by aspartic acid. This substitution results in a reduced tendency for self-association, thereby allowing a more rapid absorption from the subcutis. IAsp injected immediately before a meal therefore results in significantly reduced postprandial glucose levels compared with regular insulin (12,13).

As a consequence of their earlier onset and shorter duration of action, it is often necessary to supplement rapid-acting analogs with basal insulin to avoid late postprandial and fasting hyperglycemia (14,15). Premixed formulations of solu-

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Abbreviations: AUC, area under the curve; BHI 30, biphasic human insulin 30; BIAsp 30, biphasic insulin apart 30; DCCT, Diabetes Control and Complications Trial; DECODE, Diabetes Epidemiology: Collaborative Analysis Of Diagnostic Criteria in Europe; IAsp, insulin aspart; Mix25, biphasic insulin lispro 25; PP, per protocol.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

ble human insulin and an intermediate-acting component (NPH insulin) are commonly prescribed for insulin-requiring type 2 diabetes because of increased convenience and reduced number of daily injections. It has been estimated that ~40% of individuals with diabetes worldwide are treated with premixed insulin preparations, of which the most commonly prescribed contains 30% soluble insulin and 70% intermediate-acting insulin (16).

Biphasic insulin aspart 30 (BIAsp 30) is a premixed formulation containing 30% soluble IAsp and 70% protaminated IAsp. Previous clinical trials evaluating the pharmacodynamics of BIAsp 30 in healthy individuals have shown that the fast onset of action seen with IAsp is retained in the biphasic formulation, whereas the duration of action has been extended to match that seen with biphasic human insulin 30 (BHI 30) (17). A similar premixed formulation for another rapid-acting insulin analog has been developed containing 25% soluble and 75% protaminated insulin lispro 25 (Mix25) (18).

The primary objective of the present trial was to investigate whether postprandial glucose excursions in individuals with type 2 diabetes are reduced using BIAsp 30 compared with Mix25 and BHI 30. To test this, we compared postprandial glucose excursions over 5 h after a single premeal injection of each of the premixed insulin preparations in a controlled meal test setting.

RESEARCH DESIGN AND METHODS

Design

This was an open-label, multicenter, randomized, three-way crossover trial comparing BIAsp 30 with BHI 30 (Mixtard 30/70; Novo Nordisk, Bagsværd, Denmark) and Mix25 (Humalog Mix75/25; Eli Lilly, Indianapolis, IN) in subjects with type 2 diabetes. The trial was conducted at three sites in Denmark. BIAsp 30 and BHI 30 were provided in 100 units/ml, 1.5-ml Penfill ampules for use with a NovoPen injection device (Novo Nordisk), and Mix25 was provided as a disposable 100 units/ml, 3-ml pen system (Humalog Mix75/25 Pen; Eli Lilly), kindly provided by Novo Nordisk A/S. The trial protocol was approved by the Danish Medicines Agency and local ethics committees and was conducted in accordance with the Declaration of Helsinki (19). Written informed consent was obtained from all subjects.

Subjects

The following inclusion criteria were used: insulin-treated type 2 diabetic subjects ≥18 years of age, with BMI <32 kg/m^2 and HbA_{1c} <11.0%. Required total insulin dose at entry was <1.4 units/kg per day. Subjects with late diabetic complications such as impaired renal function (serum creatinine >150 μmol/l), cardiovascular disease (including severe uncontrolled hypertension), proliferative retinopathy, and advanced neuropathy, as well as subjects with recurrent severe hypoglycemia, impaired hepatic function, or alcohol/drug abuse, were not included in the trial.

Study day procedure

Trial procedures were performed on 3 study days separated by a washout period of at least 5 days. Subjects were randomized symmetrically to one of six possible treatment sequences by allocation of the lowest available randomization number, such that each subject received a single dose of one of the three biphasic insulin preparations on each study day in random order.

Subjects attended the trial site in the morning after an overnight fast. Oral antidiabetic agents and intermediate-acting or long-acting insulins were discontinued 24 h before each study day, and consumption of alcohol was not permitted during the 24 h before the visit. Preinjection blood glucose target levels of 6–10 mmol/l were obtained by nighttime subcutaneous injection of short-acting insulin, if necessary. A single subcutaneous injection (0.4 units/kg body wt) of one of the three biphasic insulin preparations was administered in the abdomen before eating a standard breakfast on each study day. Insulin preparations were supplied in an open-label fashion because the timing of premeal injections differs between treatments: BIAsp 30 and Mix25 were injected immediately before the meal, according to clinical recommendations (20), whereas BHI 30 was injected 15 min before the meal. The standard breakfast was the same for all study days and was consumed within 15 min. The breakfast meal contained 532 kcal (50% from carbohydrate, 35% from fat, 15% from protein).

Biochemical analysis

All biochemical analyses were performed at a central laboratory (Nova Medical Medi-Lab A/S, Copenhagen, Denmark). Serum glucose concentrations were measured using standard enzymatic methods (21). The measured serum human insulin, insulin aspart, and insulin lispro concentrations were corrected for endogenous insulin, which was estimated based on the measured C-peptide concentration in the serum (22) using Dako C-peptide ELISA (Dako Diagnostics, Ely, Cambridgeshire, U.K.). Human insulin, insulin aspart, and insulin lispro were measured with Pharmacia Insulin radioimmunoassay 100 (Pharmacia Diagnostics AB, Uppsala, Sweden). The measured serum insulin lispro was corrected for nonlinearity using a method similar to that previously described for insulin aspart by Andersen et al. (23).

Standard biochemical and hematological clinical parameters were measured at baseline.

Pharmacodynamic and pharmacokinetic assessments

For each study visit, 5-h serum glucose profiles and insulin profiles were constructed. Blood samples for determination of serum glucose were collected in 15-min intervals from 30 min before the meal to 3 h after the meal and at 30-min intervals for the last 2 h of the 5-h sampling period.

Postprandial glucose control was assessed by overall glucose excursions (EXC) over 5 h [$EXC_{0-5\ h(SG)}$], early postprandial excursions from 0 to 2 h [$EXC_{0-2\ h(SG)}$], and late postprandial excursions from 2 to 5 h [$EXC_{2-5\ h(SG)}$]. EXC was calculated as the absolute area delimited by the serum glucose concentration-time curve and baseline concentration within a time interval. Serum glucose concentration profiles were further characterized by maximum concentration [$C_{max(SG)}$] and time to maximum concentration [$t_{max(SG)}$]. The corresponding serum insulin concentration profiles were characterized by the area under concentration curve [$AUC_{0-5\ h(Ins)}$] 0–5 h after injection, maximum concentration [$C_{max(Ins)}$], and time to maximum concentration [$t_{max(Ins)}$] after injection. All areas were calculated using the trapezoidal method. Blood samples that were hemolyzed or for which negative values were derived were

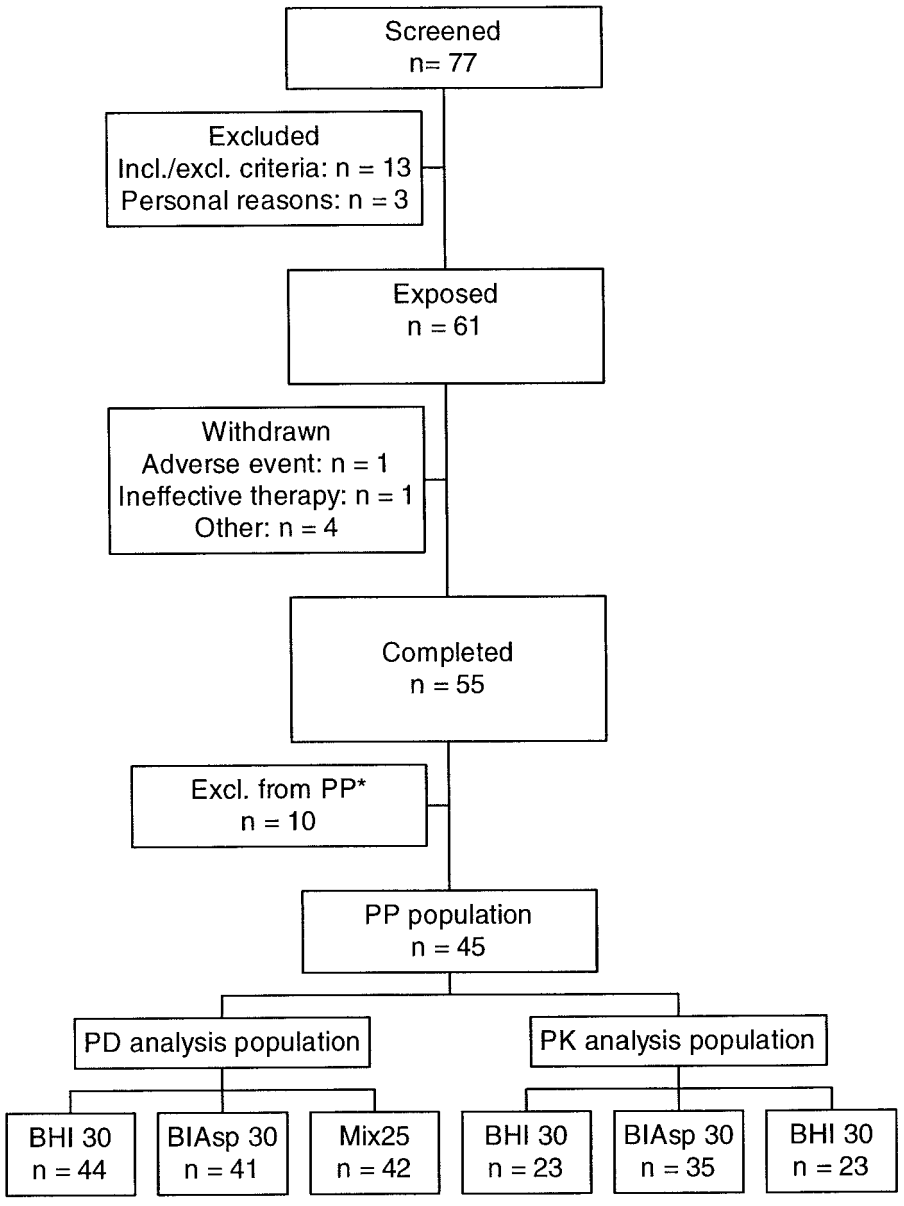

Figure 1—*Population allocation. *Subjects excluded from the PP population who violated the protocol or had pharmacodynamic and pharmacokinetic profiles for only one of the trial products.*

regarded as missing. Profiles were excluded if more than five values were missing and if baseline values were missing.

Statistical analyses

The study was designed to have 80% power (53 subjects) to detect a relative difference of 25% between treatments for the postprandial glucose excursion ($EXC_{0-5\ h(SG)}$). All analyses were based on the per-protocol (PP) population, which included all subjects who did not violate the protocol and who had acceptable pharmacodynamic and pharmacokinetic profiles for at least two of the trial products. End points were analyzed by a two-way ANOVA with treatment as a fixed effect and subject as a random effect, with a 5% level of significance. Except for $t_{max(SG)}$ and $t_{max(ins)}$, all analyses were based on log-transformed data. If observations were only available up to 4 h after the meal, the profiles were extrapolated to 5 h by applying the approach of last observation carried forward. If <4 h of data was available, profiles were excluded from the analysis. Statistical analyses were performed using SAS version 6.12 on a UNIX platform (SAS Institute, Cary, NC).

RESULTS

Subjects

A total of 61 insulin-treated type 2 diabetic subjects were randomized in the trial: 40 men, 21 women; aged 60.1 ± 9.4 years (range 35–80); BMI 27.3 ± 3.6 kg/m^2 (8.1–32.2), and HbA_{1c} 8.3 ± 1.1% (5.6–10.5%). Duration of diagnosed type 2 diabetes was 11.6 ± 6.4 years (0.5–23). One subject having BMI of 32.2 kg/m^2 was included in the trial. However, this deviation was considered of minor importance and the subject was therefore included in the analysis. One subject was withdrawn due to a serious adverse event (transient ischemic attack) after treatment with BIAsp 30, which was not related to therapy. One subject withdrew from the trial due to ineffective therapy 28 days after one of the meal tests, in which the subject was treated with Mix25. Four additional subjects withdrew from the trial for personal reasons. Of the 55 subjects who completed the trial, 45 were included in the PP analysis population (Fig. 1).

Postprandial serum glucose concentration

Mean pretest fasting serum glucose levels obtained were similar between groups (8.4–8.6 mmol/l). Postprandial glucose control, as assessed by serum glucose excursion 0–5 h after the meal ($EXC_{0-5(SG)}$), was significantly superior with BIAsp 30 compared with either BHI 30 or Mix25: 17% lower than with BHI 30 ($P < 0.001$) and 10% lower than with Mix25 ($P < 0.05$) (Fig. 2, Table 1). Glucose excursion was significantly lower during both the early (0–2 h) and the late (2–5 h) postprandial phase with BIAsp 30 than with BHI 30 (EXC_{0-2}, $P < 0.01$; EXC_{2-5}, $P < 0.01$). Glucose excursion with BIAsp 30 was also significantly lower than with Mix25 during the late postprandial phase ($P < 0.05$) but not during the early phase. $C_{max(SG)}$ for BIAsp 30 was significantly lower than that for BHI 30 ($P < 0.05$) but was not different from Mix25. Finally, $t_{max(SG)}$ was significantly shorter for BIAsp 30 than for either of the other preparations (13 min shorter than with BHI 30, $P < 0.01$; 11 min shorter than with Mix25, $P < 0.05$) (Table 1).

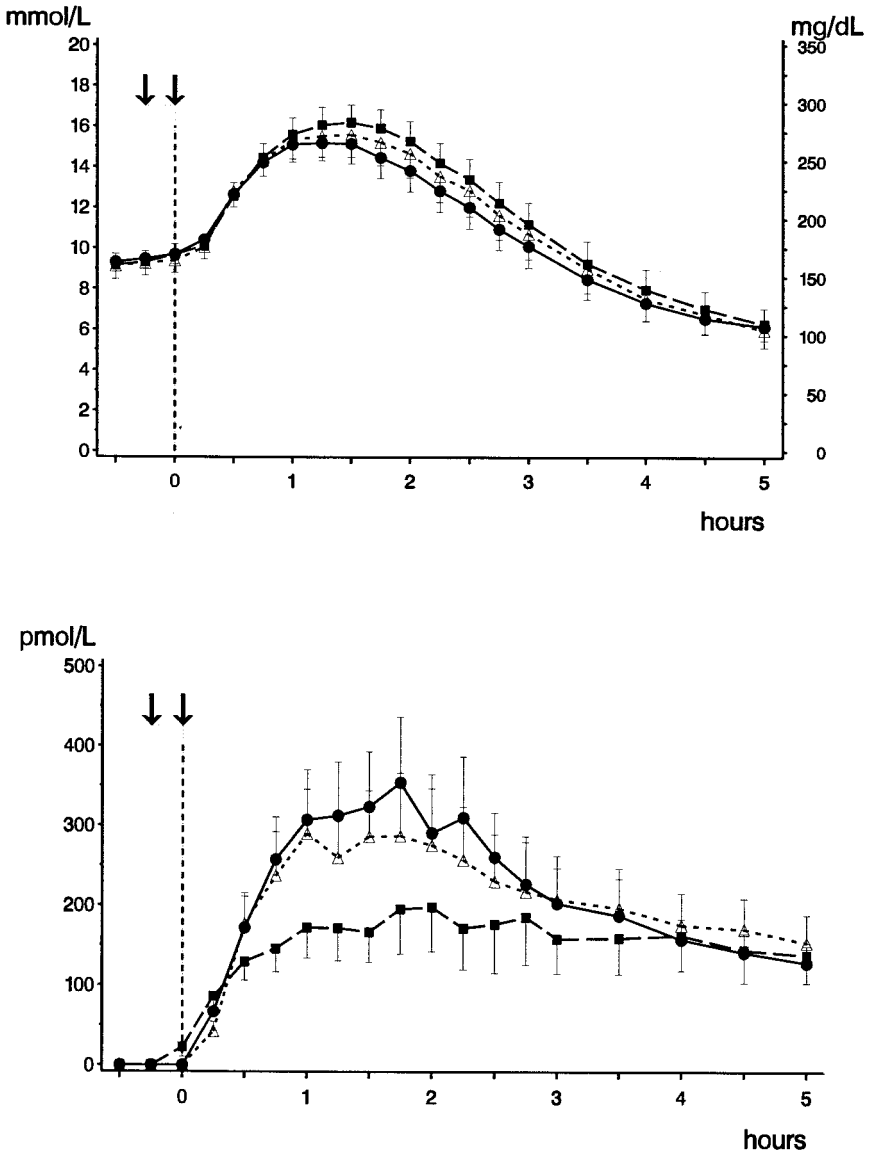

Figure 2—*Mean postprandial serum glucose (top) and insulin (bottom) profiles for subjects with type 2 diabetes (PP population) after injection of BIAsp 30 (●) and Mix25 (△) immediately before test meal, and BHI 30 15 minutes before test meal (■). Arrows indicate the injection times; vertical dotted line marks the time of meal ingestion. Error bars represent 2 × standard error of the mean.*

Postprandial serum insulin concentration

A larger $AUC_{(0-5\ h)}$ and an approximately twofold higher $C_{max(ins)}$ were seen after injection with BIAsp 30 than after injection with BHI 30 ($P < 0.001$ for both parameters). The time to $C_{max(ins)}$ was 55 min shorter for BIAsp 30 than for BHI 30 ($P < 0.001$). The $C_{max(ins)}$ was 12% higher and the $AUC_{0-5\ h(ins)}$ was 7% higher for BIAsp 30 as compared with Mix25 (Fig. 2), but this did not reach statistical significance (Table 1).

Safety

One serious adverse event and one nonserious adverse event were reported after treatment with BIAsp 30, whereas two nonserious events were reported with BHI 30. None of the events were evaluated as being related to treatment. Overall, 53 hypoglycemic episodes were reported during study days (23 episodes with BIAsp 30, 11 episodes with BHI 30, and 19 episodes with Mix25). Most were mild (symptoms only, not confirmed by blood glucose measurements) and resolved spontaneously. There were very few severe hypoglycemic episodes (requiring third-party intervention) during the trial (two episodes with BIAsp 30, two episodes with BHI 30, five episodes with Mix25), and no clinically significant abnormalities in biochemical or hematological measurements or vital signs.

CONCLUSIONS — Glycemic control as measured by HbA_{1c} does not discriminate between the relative contribution of fasting and postprandial glucose levels, and it is not clear whether therapies that target postprandial glycemia per se have specific benefits compared with other therapies that lower HbA_{1c}. No prospective studies confirming the clinical relevance of postprandial glycemic control are available (24). Such studies would not be easy to conduct, because it is difficult to distinguish between the benefits of improved postprandial control from overall glucose control. However, postprandial hyperglycemia is now recognized as being associated with the risk of developing microvascular and macrovascular complications in diabetes (7–11,25,26). Both the DCCT (1) and the Kumamoto Study (2,27) targeted postprandial glucose levels as part of their intensive treatment arms to improve overall glucose control as measured by HbA_{1c} in subjects with type 1 and type 2 diabetes, respectively. In both cases, intensive treatment with mealtime insulin was associated with reductions in microvascular complications. The importance of a high glucose response is further supported by the results of the DECODE epidemiological study, which included data from >20,000 subjects with varying degrees of abnormal glucose homeostasis (ranging from normal to diabetic). In all groups, 2-h blood glucose levels after oral glucose load were shown to be better predictors than fasting glucose levels of cardiovascular disease and death from all causes (9,10).

Although improvements in postprandial glucose control after treatment with BIAsp 30 compared with BHI 30 have previously been reported (17,28), this is the first direct comparison of the two biphasic insulin analog formulations BIAsp 30 and Mix25. The results of this trial show that postprandial glucose concentrations in individuals with type 2 diabetes can be more effectively controlled with BIAsp 30 than with either BHI 30 or Mix25. It should be stressed that the BHI

Table 1—*Results and ANOVA comparisons of BIAsp 30 (injected at mealtime) with Mix25 (injected at mealtime) and BHI 30 (injected 15 min before mealtime)*

		Ratio* between treatments (95% CI)	
Glucose end point	Means ± SD	BIAsp 30 / BHI 30	BIAsp 30 / Mix25
Pharmacodynamic end points:			
Glucose excursion			
$EXC_{0–5\ (SG)}$ (mmol/l × h)			
BHI 30	20.1 ± 4.9	0.83 (0.77; 0.90)†	
BIAsp 30	16.6 ± 4.4		0.90 (0.83; 0.98)‡
Mix25	18.9 ± 6.1		
$EXC_{0–2\ (SG)}$ (mmol/l × h)			
BHI 30	9.4 ± 2.7	0.81 (0.71; 0.93)§	
BIAsp 30	7.7 ± 2.7		0.97 (0.85; 1.11)
Mix25	8.5 ± 3.3		
$EXC_{2–5\ (SG)}$ (mmol/l × h)			
BHI 30	10.1 ± 3.2	0.82 (0.72; 0.94)§	
BIAsp 30	8.3 ± 2.6		0.88 (0.77; 1.00)‡
Mix25	9.7 ± 3.8		
$C_{max\ (SG)}$ (mmol/l)			
BHI 30	16.7 ± 2.6	0.95 (0.91; 1.00)‡	
BIAsp 30	15.9 ± 2.7		0.99 (0.94; 1.04)
Mix25	16.4 ± 3.2		
$t_{max\ (SG)}$ (min)			
BHI 30	88.0 ± 26.4	−13.2 (−22.2; −4.1)§	
BIAsp 30	75.1 ± 22.2		−11.3 (−20.5; −2.11)‡
Mix25	86.5 ± 26.9		
Pharmacokinetic end points:			
Insulin			
$AUC_{0–5h\ (ins)}$ (pmol/l × h)			
BHI 30	741 ± 426‖	1.72 (1.40; 2.10)†	
BIAsp 30	1,079 ± 535		1.07 (0.90; 1.28)
Mix25	1,031 ± 621		
$C_{max\ (ins)}$ (pmol/l)			
BHI 30	237 ± 156	2.01 (1.64; 2.46)†	
BIAsp 30	415 ± 244		1.12 (0.95; 1.34)
Mix25	360 ± 211		
$t_{max\ (ins)}$ (min)			
BHI 30	169 ± 71	−55.3 (−85.0; −25.5)†	
BIAsp 30	115 ± 59		15.15 (−11.4; 41.7)
Mix25	100 ± 41		

PK and PD parameter data are means ± SD. The ratios and differences (with 95% CIs) refer to ANOVA analyses comparing BIAsp 30 with Mix25 and BHI 30 treatments in the PP population. *Ratios except for t_{max} are difference in minutes; †$P < 0.001$; ‡$P < 0.05$; §$P < 0.01$; ‖data obtained from $t = -15$ to $t = 285$.

30 was administered 15 min before the meal. It cannot be excluded that administration of the agent 30 min before the meal would have changed the glycemic response to BHI 30 slightly. A more detailed examination of the postprandial serum glucose excursion showed that the late postprandial serum glucose excursion (2–5 h) was significantly lower with BIAsp 30 than Mix25. Whether this difference in glucose excursion between BIAsp 30 and Mix25 is due to the difference in ratios of soluble and protaminated analog, to a difference between the native analogs, or to a combination of the two is yet to be determined.

Analyses based on the pharmacokinetic profiles showed that maximum serum insulin concentrations were higher and were obtained faster with BIAsp 30 administered immediately before the meal compared with BHI 30 administered 15 min before eating. No significant differences could be demonstrated between BIAsp 30 and Mix25, despite a tendency for higher serum insulin concentrations with BIAsp 30 from 1 to 2.5 h (Fig. 2). These pharmacokinetic results should be interpreted with caution, because insulin profiles were excluded for several subjects due to hemolyzed blood samples and/or negative derived values (Fig. 1). In addition, the C-peptide corrections require a constant ratio between C-peptide and endogenous insulin, which is only the case in a fasting state. However, due to the crossover design and the fact that the meal test conditions were similar for all treatments, a possible C-peptide correction error must be considered comparable for all three treatments.

The present trial only shows results after a single meal, and limited data are presently available to establish the long-term benefits of twice-daily treatment with biphasic insulin analogs in subjects with type 2 diabetes. Improved postprandial glucose control and similar HbA_{1c} values have been shown after 3 months of treatment with BIAsp 30 compared with BHI 30 in type 1 and type 2 diabetic subjects (29). Similar results have been shown for Mix25 in a 6-month randomized trial (18).

In conclusion, BIAsp 30 provides improved postprandial glucose control in type 2 diabetic subjects when compared with Mix25 and BHI 30. Although the clinical relevance of the observed improvement in postprandial glucose control remains to be proven in prospective long-term studies, a treatment modality that tends to normalize the postprandial glycemic responses is considered likely to exert a beneficial impact on the development of long-term diabetic complications and death rates of diabetic subjects (1,5,9–11).

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